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CHEMICAL RESEARCH, DEVELOPMENT & ENGINEERING CENTER

CRDEC-TR-009

SUPPORT FOR THE DELISTING OF DECONTAMINATED LIQUID CHEMICAL SURETY MATERIALS AS LISTED HAZARDOUS WASTE FROM SPECIFIC SOURCES (STATE) MD02 IN COMAR 10.51.02.16-1

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U.S. ARMY ARMAMENT MUNITIONS CHEMICAL COMMAND

Aberdeen Proving Ground, Maryland 21010-5423

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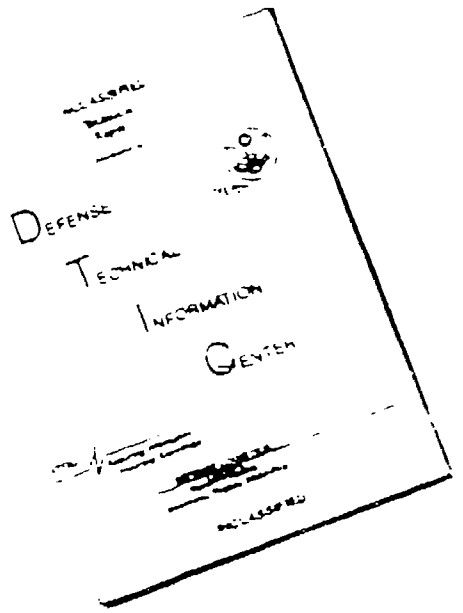

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Maryland recently enacted regulations that listed decontaminated residues of certain chemical warfare agents as hazardous wastes. The State would consider delisting if the Army document the effects of its decontamination procedures. Army specialists at U.S. Army Chemical Research, Development and Engineering Center (CRDEC), Aberdeen Proving Ground, MD, have had exhaustive experience in this area since 1918 when chemical agents were first used in combat in World War I. Competence accrued during this 70-year legacy includes destruction of laboratory and training wastes, combat decontamination, and largescale demilitarization of unserviceable and obsolete agent-filled munitions. The facts and circumstances enumerated in this document indicate that current decontamination practices are safe, scientifically valid, and result in the total destruction of agents in question. Several basic issues were addressed. (1) Do theoretical chemical calculations
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support claims that agents plus decontaminants yield products that no longer contain agents? They do. Reaction energies, reaction kinetics, chemical equilibrium, laws of thermodynamics, and other mathematical considerations indicate that A + B do indeed equal C + D. (2) Are older decontamination procedures that used different reagents equivalent to today's protocols and reagents? In most cases, yes. For example, when using hydroxide or sodium carbonate, the reactive decontaminating moiety in both cases is the hydroxyl ion. (3) Do analytical results and toxicological data substantiate complete destruction of chemical agents when decontaminated? Yes. Extensive information accrued since 1918 provides incontrovertible scientific evidence of decontamination efficacy.

EXECUTIVE SUMMARY

Maryland recently enacted regulations that listed decontaminated residues of certain chemical warfare agents as hazardous wastes. Delisting would be considered by the State were the Army to document the efficacy of its decontamination procedures.

Army specialists at Edgewood, MD (Chemical Research, Development & Engineering Center - CRDEC) have had exhaustive experience in this area since 1918 when chemical agents were first used in combat in World War I. Competence accrued during this seventy-year legacy includes destruction of laboratory and training wastes, combat decontamination, and large-scale demilitarization of unserviceable and obsolete agent-filled munitions. The facts and circumstances enumerated in this document indicate that current decontamination practices are safe, scientifically valid, and result in the total destruction of agents in question.

Several basic issues were addressed:

a. Do theoretical chemical calculations support claims that agents plus decontaminants yield products that no longer contain agents? They do. Reaction energies, reaction kinetics, chemical equilibrium, laws of thermodynamics, and other mathematical considerations indicate that A + B do indeed equal C + D.

b. Are older decontamination procedures, which used different reagents, equivalent to today's protocols and reagents? In most cases, yes. For example, when using sodium hydroxide or sodium carbonate, the reactive decontaminating moiety in both cases is the hydroxyl ion (^-OH).

c. Do analytical results and toxicological data substantiate complete destruction of chemical agents when decontaminated? Yes. Extensive information accrued since 1918 provides incontrovertible scientific evidence of decontamination efficacy.

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PREFACE

The work described in this report was authorized under Project No. 1L162706A553F, Decontamination and Contamination Avoidance. This work was started in December 1987 and completed in February 1988.

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This report has been approved for release to the public.

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SUPPORT FOR THE DELISTING OF DECONTAMINATED LIQUID CHEMICAL SURETY MATERIALS AS LISTED HAZARDOUS WASTE FROM SPECIFIC SOURCES (STATE) MD02 IN COMAR 10.51.02.16-1

1.0 INTRODUCTION

1.1 REGULATORY BACKGROUND

In January 1986 the State of Maryland enacted regulations that identified certain chemical warfare agents (also known as chemical surety materials - CSM) as hazardous wastes. Included were nine listed waste solutions identified in the regulation as the following: Industry, Military; EPA Hazardous Waste Number K991-K999. According to the State, these decontaminated residues were included to make it clear that treated wastes were of concern.

Personnel from the U.S. Army Chemical Research, Development and Engineering Center (CRDEC) noted that decontamination procedures convert the chemical agents in question to non-surety products, and requested guidance in addressing this issue.

The State offered that if CRDEC personnel document that decontaminated residues contain no detectable levels of CSM, this information could be used as a basis to consider delisting the waste residues. Specifically, the State asked that CRDEC:

a. Provide a detailed description of actual decontamination processes including a step-by-step outline of each procedure, identification of the decontaminating agent used on each agent, the theoretical chemical reaction, the concentration of decontaminant used, amount of time each reaction is allowed to proceed, plus any parameters that influence the degree to which a reaction goes to completion.

b. Describe procedures which assure that solutions used to perform toxicological tests are equivalent to solutions which result from the actual decontamination procedures.

c. Describe the protocol for toxicological testing in order to determine whether it follows generally accepted practices.

This document has been prepared by CRDEC for review by State of Maryland and other regulatory officials to assure that current standard decontamination procedures result in wastes which can be excluded from regulations as hazardous.

1.2 INSTITUTIONAL HISTORY

The agency now known as CRDEC has been located on Gunpowder Neck peninsula in Harford County, Maryland since 1918 when the land was bought by the War Department. The impetus for this purchase was the unprecedented and devastating use of chemical warfare agents during World

War I (Allied forces suffered more than one-third of their casualties as a result of chemicals). The basic mission--defense against chemical and biological agents, and providing a chemical retaliatory capability--has remained unchanged for more than seventy years.

CRDEC is unique among military entities: it is one of the very few that has Joint Service responsibilities. In other words, it executes its mission on behalf of the Army, Navy, Air Force, and Marines. All U.S. fighting forces thus depend entirely on CRDEC to provide the decisive edge in combat on a contaminated battlefield. Major support includes: gas masks and filters for armored vehicles and buildings; protective clothing; decontamination formulations and devices; methods to avoid contamination such as self-decontaminating coatings and surfaces; detectors and alarms; and retaliatory chemical munitions should deterrence fail.

Therefore, CRDEC scientists, engineers, and technicians have dealt continuously and for more than seventy years with all aspects of chemical agents and munitions, have accrued a record of laboratory safety, and have become uncontested leaders in innovative research and development as well as proper decontamination of chemical surety materials in the Western World.

This legacy, reflected in bibliographical citations, reaches back to 1918 - e.g., "The Cleaning of Objects Contaminated with Yperite [Mustard]", Chemical Warfare Service Report No. Z-197, May 1918, Washington, D.C., and "Solubility and Rate of Hydrolysis of Mustard Gas in Water", R. E. Wilson, *et al.*, *Journal of the American Chemical Society* **1922**, *44*, 2867. The point is that CRDEC scientists and engineers are free-world experts in decontaminating chemical warfare agents.

Extensive decontamination experience and comprehensive data bases have underwritten huge demilitarization projects in the past including GB-filled M55 rockets, and M139 and E139 bomblets at Tooele Army Depot, Utah. In all cases, decontamination and disposal projects for agent-filled munitions were executed safety, without untoward incident, and in total compliance with every prevailing environmental and human safety requirements and concern. These and other facts enumerated in detail in this document provide ample evidence that current decontamination protocols and procedures are safe, scientific, and result in the total destruction of chemical agents.

1.3 BASIC ORGANIZATION OF THE DOCUMENT

To be responsive to the State's requests, this manuscript has been organized into the following general areas:

(1) The theoretical chemistry basis for asserting that agent plus decontaminant yields less-hazardous products. Included in this section are discussions of the agents listed, decontamination operational definitions (i.e., how clean is clean?), decontamination procedures, theoretical reaction

mechanisms and kinetics involved, and theoretical bases for analyzing reaction products.

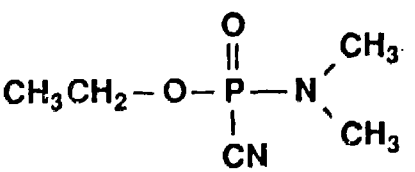
(2) The issue of equivalence in chemical-agent decon. For example, if an older, standard decontamination procedure was based on using sodium carbonate and the modern version prescribes sodium hydroxide, one might conclude that there is no scientific basis upon which to compare results. On the contrary, the active decon moiety in both cases is the hydroxyl ion (^{-}OH), and the agent being decontaminated reacts exactly the same.

(3) Archival data. Since 1918, information has accrued about decon efficiency from sources as varied in scope and complexity as sophisticated laboratory experiments, combat operations, training exercises, field trials, and wholesale destruction of unserviceable munitions. The data used here are in two forms, analytical and toxicological, and provide a comprehensive foundation upon which to structure conclusions of efficacy.

(4) Analytical methods. A review of methods utilized to determine the concentration of active material before and after the decontamination process. Included are the most recent literature from both CRDEC and other agencies involved in Decontamination methodology.

2.0 Decontamination

2.1 Agents (State of Maryland Listing)



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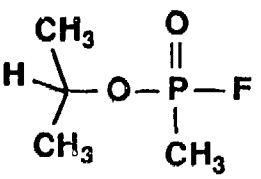

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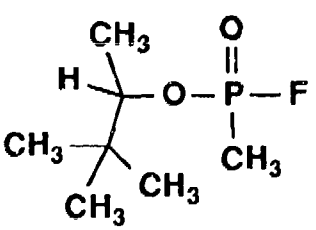

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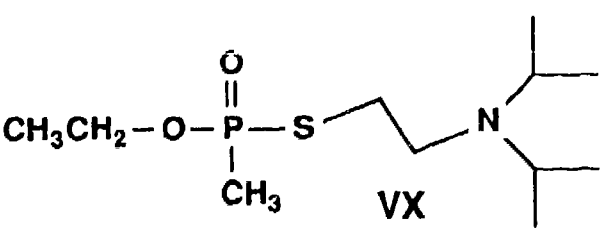

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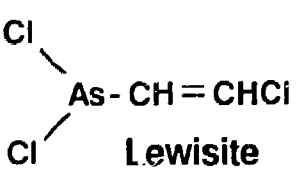

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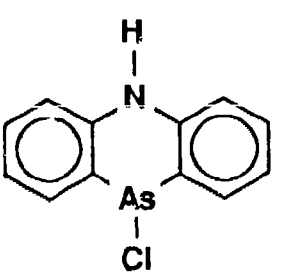
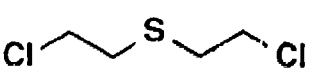
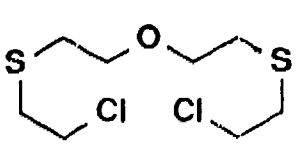


Adamsite



HD - (Mustard)



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2.2 Decontamination Operational Definitions

Over the years, several reviews have been published concerning chemical warfare (CW) agent decontamination. These reviews have generally focused on fielded and/or experimental systems which have been used to detoxify agents. But, what is decontamination? The answer, like that to the question "What is clean?", is not simple or direct. In field expedient decon, the procedure may be anything which delays the toxic effects of an agent, to a level below which the problem may be ignored. The purpose of such a decon action is to reduce the need for wearing maximum individual protective gear and/or to reduce the likelihood of exposure to agent. Deliberate decon, in contrast, is administered under controlled conditions and has at its basis removal from the environment of the maximum amount of the toxic material - - in the best case not only below toxic levels but below all detectable concentrations. Its goal is to remove all contamination so that equipment may be returned to service or sent to a maintenance facility without presenting a hazard to unprotected personnel.

Decontamination by purely physical processes is thus undesirable as it does not solve the problem, but only moves it to a different location. Use of a chemically reactive system which can convert the CW agents to non-toxic materials remains the most viable approach. Once it has been decided to chemically react, and thus destroy, an agent, questions immediately arise concerning the level of chemical destruction and kinetics and products produced. In deliberate decon a minimum requirement is that 10 half-lives of destruction to less-toxic products must be obtained during 10 minutes at room temperature. Starting with this minimum requirement, the chemistry described below is aimed at increasing the speed of destruction and the control of the products to the lowest possible level to ensure no toxic exposure after decontamination. This usually means that decontamination is not considered complete unless greater that 99.9% destruction to less-toxic products is certified.

2.3 Theory of DECON Procedures

2.3.1 Introduction

Chemical equations depict reactions between molecules. They conventionally are written to show initial reactants and final products; i.e., A + B $\rightarrow$ C + D.

But this is the most elementary sort of scientific stenography, for confounding arrays of conditions and factors exist within the molecular milieu, however, simplistically transcribed.

For molecules to react, particles (e g., agent and decontaminant) must collide, and these collisions must result in interactions between particles. The Laws of Thermodynamics decree that reactions proceed from higher to lower energy states. Factors that influence the rate of a chemical reaction include the

nature of reactants, amount of contact area, concentration of reactants, temperature and, in some cases, presence of a catalyst.

Other factors are involved in determining the degree of completeness of chemical reactions. In many instances, time is of obvious importance. Some reactions achieve an equilibrium state in which A + B and C + D are present in discrete concentrations. If, for example, compound "B" is a chemical warfare agent and a state of equilibrium has been reached whereby some "B" remains, the conversion of "B" can be driven to completion by adding an excess of reactant "A" (or, conversely, by removing some of the products "C" and "D"). This effect of concentration on chemical equilibrium was succinctly summarized by Henri Le Châteiier (1888): "Any change in one of the variables that determines the state of a system in equilibrium causes a shift in the position of equilibrium in a direction that tends to counteract the change in the variable under consideration." In the same way, the rate of many slow decon reactions can be accelerated by adding excess concentrations of decontaminant.

Phase separations (e.g., physically resembling oil on water) can cause reaction rates to be painfully slow because agent and decon molecules react only at the interface. Effective mixing is normally employed to solve this problem.

Other factors (such as pH or side reactions) can also influence the ultimate objective of any decontamination exercise: that is, the complete destruction of a chemical agent.

In the following sections of this document, the theoretical basis for each decontamination reaction is elucidated.

The theoretical treatment comprises three basic approaches:

a. Thermodynamics (2.3.2). The reactions described should work because all are going "downhill" from higher to lower energy states.

b. Kinetics (2.3.3). Rate constants, half-lives, and other mathematical expressions are employed to calculate how fast decon reactions proceed.

c. Product analysis (2.3.4). In this "materials balance" section, predictions of chemical structures and amounts of products are calculated based on the assumption that 100 percent of the chemical agent is converted to products.

2.3.2 Thermodynamics of DECON Procedures

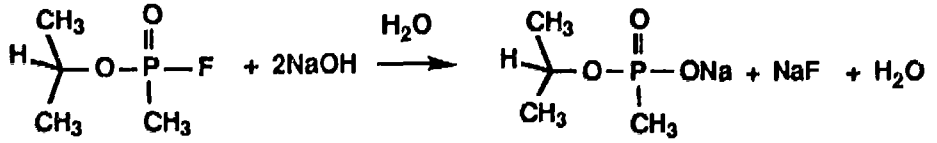
In the reaction between an agent and a decon solution, several possibilities must be considered. Using the base hydrolysis of GB as an example, three plausible events may occur to affect the net amount of GB in the Decon solution:

1. The neutralization reaction may proceed, reducing the GB concentration.

2. Sodium isopropyl methylphosphonate may be reconverted to GB (reformation of agent).

3. GB may evaporate from the reaction mixture and be vented into the environment.

We first consider possibilities 1 and 2 together. The reaction of GB with hydroxide, shown below:



is highly exothermic, with a free energy of about -30 kcal/mole. The heat generated by this reaction is such that precautions must be taken to prevent overheating of the reaction vessel in bulk decon procedures. The rate of this reaction increases with increasing temperature and pH. Since the reaction mixture and the neutralized brine contain an excess of base (NaOH or Na_2CO_3), any evaporation of water from the brine will increase the pH of the solution and hence speed the reaction. Thus the equilibrium constant of the reaction can be calculated from the free energy of the reaction:

$\Delta G = -2.303\ RT \log K_{eq}$

where R is the gas constant, ΔG the free energy, T the absolute temperature and K_{eq} the equilibrium constant. In the decon reaction the temperature is generally close to room temperature (25°C, 298 K), so:

$-30{,}000\ \text{cal/mole} = -2.303 \times 1.987\ \text{cal/mole}\cdot{}^{o}\text{K} \times 298\ \text{K} \times \log K_{eq}$,

or $\log K_{eq} = 21.9$. Thus in theory the conversion of GB to sodium isopropyl methylphosphonate should be nearly complete.

Epstein, *et al.* (1977) discussed the possibility of reconversion of sodium isopropyl methylphosphonate to GB in the presence of HF or other acids in the vapor above the salt solution. Two requirements for this reaction are an acidic environment and an absence of water to shift the equilibrium of the reaction to formation of GB (neither condition exists in the normal liquid decon procedures). However, even if this reaction does in fact occur, the "new" GB would have two fates: reaction in the basic salt solution, or venting into the atmosphere. The first possibility is the initial reaction discussed above and reformation in solution would again subject GB to reaction with NaOH.

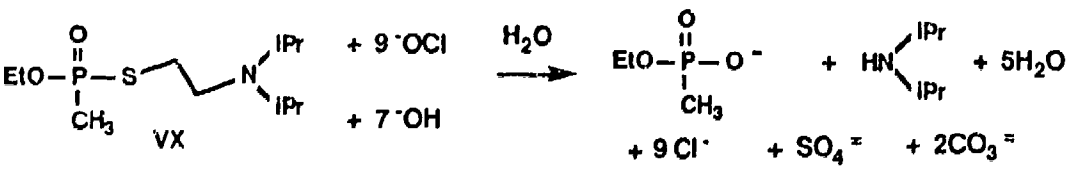
Considering the second possibility, the temperature of the decon solution is 25°C, much lower than the boiling point of GB (148°C). From the heat of vaporization of GB, we may calculate the change in its vapor pressure as a function of temperature by applying the Clausius-Clapeyron equation:

$$p = p_0 e^{(-\Delta Hvap/RT)}$$

where p is the vapor pressure, p_0 is a constant, and $\Delta Hvap$ is the heat of vaporization, presumed constant over temperature. Using $\Delta Hvap$ = 11.9 kcal/mole the vapor pressure of GB is 2.2 mmHg at 25°C (298 K).

At room temperature, considering the vapor pressure and the equilibrium constant calculated above, it is unlikely that vaporization would occur. Neither reformation of "new" GB nor vapor buildup appear to pose a hazard. GB was used in this example because of the listed agents it is the most volatile and would be the most likely to vaporize from the reaction media, if that were of concern.

Another example of thermodynamics is illustrative. The recommended procedure for decontamination of VX is reaction with alkaline hypochlorite (usually HTH, calcium hypochlorite). Compounds isolated and/or identified from reactions of VX and bleach solutions are calcium sulfate, diisopropylamine, and ethylmethylphosphonic acid. Based on these observations, the equation for the reaction of VX with alkaline hypochlorite solutions is:



With HTH, the anions precipitate as the calcium salts.

The heats of reaction, according to the equation above, can be calculated from bond energies, heats of formation and heats of neutralization to be 685 kcal/mole (Epstein, 1973). Laboratory determination (small scale - very dilute solutions) of this reaction gave 471 ± 3 kcal/mole. Larger scale reactions (similar to actual bulk decon procedures) gave a value of 675 ± 13 kcal/mole, a figure close to the theoretical value from bond energies. If any of the three values, one from calculation and the other two from experimental data, are substituted into the equation used to calculate the equilibrium constant (see GB discussion above) the conclusion is that tremendous energy is released in this oxidative destruction of VX and the equilibrium lies dramatically toward products under the conditions of the decon reaction.

Thus the calculations above suggest that the equilibrium constants for the reaction of GB with caustic (hydrolysis) and VX with hypochlorite (oxidation)

should favor the hydrolysis product by a very large margin and that reformation of "new" agent from the reaction products is negligible under standard decon conditions. Similar energetics exist for all the listed agents.

2.3.3 Kinetics of DECON Procedures

The fact that the driving force for a reaction is large (ΔG is a large negative quantity) does not mean that the reaction will necessarily occur under any given conditions. An example related to combustion is the mixture of hydrogen and oxygen at room temperature. For the reaction,

$$H_2 + 1/2O_2 \rightarrow H_2O$$

the free energy is -54.64 kcal/mole. Despite the large negative free energy term, the reaction mixture may be kept safely for decades without detectable reaction. However, a pinch of platinum-sponge (catalyst) causes the mixture to react violently (i.e., explode). The necessary affinity for reaction certainly exists in this system (thermodynamics), but the rate of attainment of equilibrium (chemical kinetics) depends on different factors.

Numerous other examples of this situation exist. Magnesium and aluminum oxidize with a very large free energy change (in excess of 100 kcal/mole). At room temperature the small film of oxide which forms on these metals makes further reaction extremely slow (thus allowing the use of these metals in structural environments). The equilibrium condition is never reached in our lifetime - - the usual time frame of importance. Incendiary bombs and the thermite reaction, on the other hand, are reminders that a large free energy in this reaction is a valid measure of the enormous affinity of the reactants to transform themselves to products.

Knowledge of the rapidity at which a reaction attains equilibrium is thus separated from the energetics of the overall reaction.

Decomposition of agent (again, for example GA) in aqueous or largely aqueous media should follow a rate law of the form:

$$\text{rate} = k_{hyd}[GA] + k_{OH}[OH^-][GA] + k_{CAT}[CAT][GA]$$

$$= \{k_{hyd} + k_{OH}[OH^-] + k_{CAT}[CAT]\}[GA] \qquad (1)$$

where the $k_{hyd}[GA]$ represents the hydroxide-independent "background" reaction, the $k_{OH}[OH^-][GA]$ represents the second order reaction between ^-OH and GA, and the $k_{CAT}[CAT][GA]$ term is the rate enhancement resulting from the addition of catalyst to the system.

Hydrolysis reactions are usually run where both water and hydroxide are in large excess. Under these conditions the first two terms of equation (1) ($k_{hyd} + k_{OH}[OH]$) are constant. If when catalyst is also in large excess over substrate ([CAT]>>[GA]) (or if catalyst is not consumed in the reaction), then the third term

is also essentially constant. Under these conditions equation (1) reduces to a description of an experimentally first-order process:

$$\text{rate} = k_{obs}[GA] \quad (2)$$

where

$$k_{obs} = k_{hyd} + k_{OH}[OH^-] + k_{CAT}[CAT] \quad (3)$$

Since in this example the system is restricted to hydroxide (no catalyst added), the term $k_{CAT}[CAT]$ reduces to 0 and the overall observed rate may be expressed as:

$$k_{obs} = k_{hyd} + k_{OH}[^-OH] \quad (4)$$

Equation (4) forms the basis of the kinetic analysis. Experimental data are plotted as k_{obs} vs. [OH]. Experimental plots of agent hydrolysis are consistent with the linear relation predicted by equation (4). A linear least-squares routine is used to determine the statistically most valid slope (k_{OH}) and intercept (k_{hyd}, no added hydroxide) for each experiment to determine the best data set. Computer analysis on each data set is then performed to compare the experimental data with a theoretical analysis based on assumption of a first order kinetic process. If the experimental values lie on the curve predicted by the assumed first order fit, then this is strong indication that the process is indeed acting as a first order kinetic process. Such experimental data are usually collected for five or more half-lives (*i.e.*, >96% reaction) to ensure good statistical analysis.

What is the value of this type of analysis? First, in the description of a kinetically first order process, the half-life of reaction ($t_{1/2}$) is independent of the concentration of agent, and is expressed as follows:

$$t_{1/2} = (\ln 2)/k_{obs}$$

Thus a measured half-life at high agent concentrations (experimentally easy to measure) is valid for agent destruction when the concentration of agent is low (experimentally difficult to measure). In a first-order reaction, it takes just as long to reduce the reactant concentration from 0.1 mole per liter to 0.05 mole per liter as to reduce it from 10 moles per liter to 5 moles per liter.

A graphic example of the predictive power of the first order kinetic condition is shown in the following table. We assume, in this example, an "agent" whose molecular weight is 100, and where we start to decon a solution of 100 g agent/L in excess aqueous hydroxide:

Initial Quantity (g)	Half-Lives	Quantity Remaining (g)	% Destroyed
100	0	100	0
100	1	50	50
50	2	25	75
25	3	12.5	87.5
12.5	4	6.25	93.75
6.25	5	3.125	96.875
3.125	6	1.5625	98.4375
1.5625	7	0.78125	99.21875
0.78125	8	0.390625	99.609375
0.390625	9	0.1953125	99.804687
0.1953125	10	0.0976562	99.902343
0.0976562	11	0.0488281	99.951171
0.0488281	12	0.0244140	99.975585
0.0244140	13	0.012207	99.987792
0.0122070	14	0.0061035	99.993895
0.0061035	15	0.0030517	99.996946
0.0030517	16	0.0015258	99.998471
0.0015258	17	0.0007629	99.999233
0.0007629	18	0.0003814	99.999614
0.0003814	19	0.0001907	99.999804
0.0001907	20	0.0000953	99.999899

[Remaining agent, column three, may be calculated from the formula $100/2^n$ which is the fraction of agent remaining, when starting with 100g, after n half lives. In general, the agent remaining when subjected to a first-order kinetic rate pattern is initial quantity/2^n, the fraction remaining after n half-lives.]

It can be seen that even when starting from a very concentrated solution the reduction in material is significant by 10 half-lives (99.9% destruction) and even more so at 20 half-lives (99.9999%).

Another value of this kinetic treatment is the determination of the "second order rate constant", k_{OH}, for the reaction. If we assume that the background hydrolysis rate is small (experimentally verified), then the major contribution to the overall rate is the hydroxide-dependent part of the reaction. This "second order rate constant" allows the calculation of the observed first order rate if the concentration of hydroxide is known. For example, if the second order rate constant between GA and hydroxide is 7.5 $M^{-1} \cdot s^{-1}$, and the concentration of hydroxide is 0.01 M (equivalent to a pH of 12), then the following calculation may be performed:

$k_{obs} = k_{OH}[OH^-]$

$k_{obs} = [7.5\ M^{-1} \cdot s^{-1}][0.01 M] = 0.075/s$

Once the observed rate constant is calculated, then the half-life of the reaction may be calculated using the relationship:

$t_{1/2} = (\ln 2)/k_{obs}$

$t_{1/2} = (0.69)/0.075/s = 9.2$ seconds

This calculation thus allows us to state that in 92 seconds (1.53 minutes, 10 half-lives) in 0.01 M hydroxide we will have destroyed 99.9% of the initial GA present in the decon solution. In 184 seconds (3.06 minutes, 20 half-lives) in 0.01 M hydroxide 99.9999 % of the initial GA will be reacted.

Once the "second order rate constant, [k_{OH}]" is known, the half-life at any specified hydroxide concentration may be calculated. Thus if the pH is raised from 12 to 13, the hydroxide concentration should be raised from 0.01 M to 0.1 M. From the equations above it can be predicted that the destruction of GA would proceed with a half-life of about 1 second, and that after 1 minute the concentration of GA would be below the ppt level.

Representative "Second Order Rate Constants" for Hydrolysis of Nerve Agents

Substrate	k_{OH} ($M^{-1} \cdot s^{-1}$)	$t_{1/2}$ (sec) at pH = 12
GA	7.5	9.2
GB	25	3
GD	10	7
VX	0.083	835

(Note: In the kinetic analysis there are three terms, the last being a catalytic term. In the usual decon solutions only hydroxide is used; however, there are catalysts which are known to accelerate the hydrolysis rate over the one observed related to base concentration. In field expedient decon this allows the reaction to proceed rapidly at lower pH's. This is of great practical interest when deconning sensitive materials. The kinetic analysis is developed to analyze the effect of added catalyst, if present in the decon system.)

2.3.4 Products of DECON Procedures

There are a number of commonly used methods for determining material balance criteria in decontamination reactions. In most cases one method is not sufficient, and the problem is generally approached from several directions. Obviously any mechanism proposed for a transformation must account for all products obtained and for their relative proportions, including products formed by side reactions. A proposed transformation cannot be correct if it fails to predict the products in approximately their correct proportions.

Traditionally the most satisfying method used to handle the mass balance problem has been to isolate the products involved. This technique is powerful but fraught with difficulties: first, large concentrations of materials must generally be utilized to ensure good isolation; second, there is always the concern that the major products of the reaction will be identified but minor products will be missed. For example, assume a hypothetical decon reaction produces two acids, acid A in 90% and acid B in 10% yield. Crystallization is a common method used to isolating acids. In the crystallization process, molecules gradually deposit from solution and attach to each other in an orderly array known as a lattice. As the aggregates of molecules grow large enough to be visible, they appear as crystalline materials. The high symmetry of these macroscopic aggregates suggests the ordered arrangement of the crystal lattice. Molecules which do not have precisely the same kinds and arrangement of forces cannot be held in the lattice. Smaller or larger molecules of similar structure are thus excluded; i.e., in the isolation of acid A, acid B will probably be excluded by the forces active in the crystallization process. Acid B will therefore be missed in the overall study. All direct isolation methodology suffers from this consideration.

In many reactions, intermediates between starting material and products are proposed. Identification of a possible intermediate is critical since an intermediate, although present in small quantities, may have toxic attributes, the final product lacks. Numerous ways, none foolproof, are used to learn whether or not a proposed intermediate is present and, if so, its structure. This problem can be subdivided into several areas. The intermediate can be isolated if it is sufficiently stable. If this isolated intermediate can be shown to proceed to products when subjected to the reaction conditions, strong evidence thereby exists that the reaction proceeds through such an intermediate. If isolation of the intermediate is unsuccessful, some spectral technique such as infrared (ir), nuclear magnetic resonance (nmr), etc., may be used. These are extremely powerful procedures which give a direct measure of the quantity and structure of an intermediate. A third variation traps the intermediate by an externally added trapping agent. In the last variation the proposed intermediate is independently synthesized then subjected to the reaction conditions thus demonstrating the products are formed. All of the techniques for determination of an intermediate lends credence to the suggested transformations of starting material to products.

Several other methods, used in conjunction with one another, provide information on product distribution. For example, isotope labeling of starting materials allows the path of reaction-to-products to be traced even under very dilute conditions. Historically use of the radioactive isotope carbon-14 has shown the power of this technique, but recent advances in analytical methods allow the use of stable (non-radioactive) isotopes in this regard.

Kinetic evidence is an extremely powerful technique in the identification of mechanism and material balance. The question being asked is: "Does the rate of disappearance of starting material equal the rate of appearance of an

identifiable product?" If this correlation can be clearly shown it is a powerful indicator of the material balance of the reaction.

As stated in the introduction, no one technique is clearly useful in all cases, but a combination of techniques draws on the power of each method. One difficulty when examining the literature of decontamination reactions is that many products of the decon reaction are difficult to analyze by traditional analytical techniques. As a result much archival information is based on the kinetic argument involving disappearance of reagents and a hypothesis of products, based on the kinetic evidence available. It has only been in the last decade that analytical techniques have been developed which allow the chemist to directly observe the products of these reactions. Use of these techniques has consistently confirmed earlier hypotheses based on kinetic analysis.

Several examples are illustrative in this regard. In the early 1980's it was discovered at CRDEC that enzymatic catalysts existed for the destruction of toxic nerve agents. Analytically the reaction was monitored by the appearance of fluoride using a fluoride ion electrode. The hypothesis of hydrolysis indicated that for every GB molecule hydrolyzed only fluoride was released. When the rate of disappearance of GB was correlated with the appearance of fluoride the rates were mirror images of one another. Although the isopropyl methylphosphonic acid was not directly analyzed, a strong implication existed that it was the only reasonable product, other than fluoride, in this decon reaction. Why then was there no direct analysis for isopropyl methylphosphonic acid to prove it is a product of the reaction? The answer lies in the analytical techniques available. Enzymatic reactions are generally run in dilute aqueous solution, and the analytical tools for directly observing isopropyl methylphosphonic acid are not as sensitive as those which detect fluoride ion. Essential in our mass balance criteria is the equation of a known amount of GB introduced into the reaction. Its disappearance is followed thus demonstrating that a known amount of fluoride is produced from the reaction mixture by equivalent rates.

Mustard hydrolysis is another case in point. Mustard dissolves in water to produced HCl. Traditionally accurate methods have been available to measure acid concentration (and, subsequently, chloride concentration). The disappearance of mustard is thus correlated with the appearance of HCl. It is encouraging that indirect kinetic studies have so often proven accurate. The classical paper on mustard reactivity of Bartlett and Swain (1946), based the mechanism on kinetic arguments and a small quantity of product isolation. It was not until the advent of nmr technology in the 1980's that direct identification of the information published in 1946 could be made. In most cases the advent of modern analytical tools has supported the suggestion in the archival literature.

We should briefly touch again on the difficulty encountered when seeking accurate data on the products of decon reactions. Many experimental techniques for accurate analysis, e.g., gc-ms, require that an aqueous reaction

media be introduced into the gas phase, then flashed, under high vacuum, into the analysis unit. The conditions of this analysis are grossly different from the conditions which existed in the aqueous decon solution. Thus, there is always concern that the analysis is not truly indicative of the situation in solution.

One extraordinary powerful technique which has recently become available to the chemist is nuclear magnetic resonance (nmr) spectroscopy. A recent development, even by chemical standards, the first nmr signals were observed in 1945 by Felix Bloch at Stanford (octane) and Edward Purcell at Harvard (water). The three-line spectrum of ethanol was reported in 1951, and in 1953 Bloch and Purcell shared the Nobel prize for their discovery. By that year, Varian Associates had delivered three nmr machines to Exxon, DuPont and Shell.

It is known that a moving electric charge creates a magnetic field. Atomic nuclei, which are known to have a charge, should also create a magnetic field if they spin. Many isotopes have what appears to be a mechanical spin, to which a spin angular momentum is assigned. All microphysical systems are quantized, and it is the spin number, i.e., the maximum observable angular momentum for the nucleus, which concerns us. For purposes of this discussion, it will suffice to say that certain nuclei exhibit this property. For example, ^{1}H, ^{13}C, ^{15}N, ^{19}F, and ^{31}P all have spins of 1/2. Frequently encountered nuclei which have no spin are ^{12}C, ^{16}O, and ^{32}S.

Every isotope with a spin not equal to zero will be characterized by a nuclear magnetic moment, which is represented by a symbol μ. This can be thought of as a bar magnet with a strength μ. If the nucleus (bar magnetic) is placed in a magnetic field, there will obviously be an interaction. Like a bar magnet, the nucleus must be either attracted to or repelled by the magnetic field. Since only two possibilities exist for a system with a spin of 1/2, there are only two possible orientations in the magnetic field, referred to as plus and minus. Thus it is clear that the nmr method requires a magnetic field as well as an external energy source. The result of some simple mathematics (not discussed here) reveals that an energy transition from a minus to a plus state may be measured. It is relatively easy to conceptualize what happens in the nmr experiment. In the absence of a magnetic field, the nuclear spins are randomized in all possible directions. When a magnetic field is applied, the spins tend to be oriented either in the same direction as the applied magnetic field (low energy state) or opposite to it (high energy state). As the molecule encounters incident radiation, energy absorption occurs and one of the spins flips direction. This energy absorption is what the nmr system detects.

The discussion of nmr theory given above only requires that the nucleus of an atom have a magnetic moment for observation of the nmr phenomenon. Large numbers of nuclei contain a magnetic moment and are thus candidates for the nmr experiment. Nuclei commonly dealt with by organic chemists which give an nmr signal under appropriate conditions include ^{13}C, ^{31}P, ^{19}F, ^{15}N, and even such ions as ^{23}Na.

The insensitivity of early instruments presented problems for nmr spectroscopy. Although many elements can be considered in the nmr technique, only nuclei which give strong signals (hydrogen, fluorine) and/or are present in the sample in high molecular concentration are practical to measure. The stable natural carbon isotope ^{13}C is present in 1.1% abundance (^{12}C has no magnetic moment). A very sensitive measuring technique is needed to determine the signal from these atoms. Recent instrument advances of the last decade have produced great sensitivity which allows routine measurement of ^{13}C spectra on normal samples. The same is true for ^{31}P spectra. In addition, both types of spectra cover a large range in the energy spectrum (i.e., ^{13}C signals range from 0 - 250 parts per million).

This technique, **because it directly observes the nuclei of an individual molecule can provide not only information of the disappearance of starting material but the appearance of product in the same experiment**. Most toxic agents contain phosphorous (^{31}P) which as indicated above gives an nmr signal. From the signal position of the phosphorous atom the group which surrounds it can be determined. In the hydrolysis of GB, the ^{31}P signal can be measured in the starting material, then watched for the shift in the ^{31}P signal to a new position as the decon reaction proceeds. It is extremely unlikely that two completely different compounds will produce the same signal in this technique. Thus, a direct non-destructive probe into the decontamination reaction is possible by watching the shift in various atoms in the starting material and products. In general, these measurements confirm literature suggestions in the archival literature. However, this is a direct observation of products under the decon solution and a certification that the toxic starting materials do not exist in solution, within the limits of sensitivity of the technique. [Note: usually anything in excess of 0.5% will be detected using this technique. Therefore a more accurate statement would be that the concentration of starting material has decreased to a level less that 0.5% of its initial value, or that the starting material is 99.5% destroyed. Specialized techniques, not discussed here, allow the nmr technique to measure down to limits of 0.01% under special circumstances.]

The time frame of the nmr experiment also permits a crude confirmation of the kinetic data discussed in 3.3.3. In other words when GB is subjected to Na_2CO_3 hydrolysis under the approved SOP, ^{31}P measurements in the nmr confirm that the only product observed is isopropyl methylphosphonic acid, and that this product is identical to the product of sodium hydroxide hydrolysis. This nmr experiment measures the chemical equivalence of these two decon procedures and ensures that this reaction goes to the indicated products to greater than 99.5%.

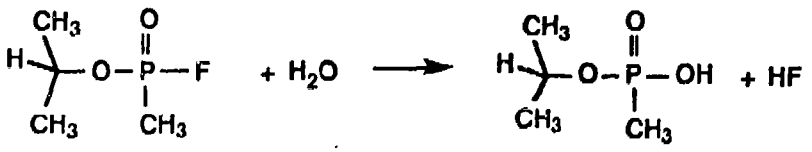
The above discussion indicates that in all the reactions under consideration the following information is available. (1) There exists an enormous thermodynamic drive to convert these toxic materials into non-toxic products. (2) Not only is there a large energetic drive to these reactions, but there is also a rapid kinetic mechanism for these transformations. (3) The product analysis discussed above demonstrates that the starting materials have

been indeed destroyed and the products clearly identified as those suggested in the archival records.

3 Equivalence of DECON Procedures

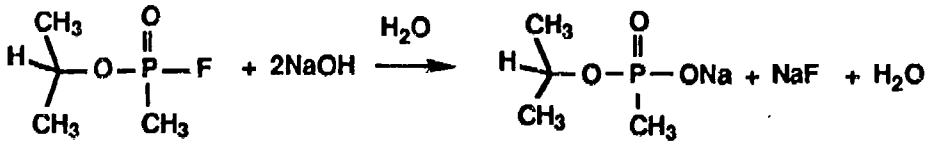
A review of the archival literature available from 1917 to the present day clearly indicates the two major chemical procedures effective in the destruction of toxic chemical agents are hydrolysis and oxidation. Within these two broad categories, however there appear numerous reagents suggested in various decon procedures. Although, at first glance, these procedures appear to be different, on close examination there are fundamental similarities in the active chemical principles responsible for decontamination. In other words, although the suggested procedures require different decontaminating agents (usually chosen for compatibility with various materials to be cleaned), the reactive species responsible for the decontamination are the same. An excellent example of this chemical equivalence is found in the base-catalyzed hydrolysis of the nerve agent GD.

In general, the term hydrolysis is utilized to indicate the addition of water to a reactive molecule with the elimination of some fragment, after the addition of water, into the aqueous solution. In the example under discussion, water will react with GB to produce one mole of hydrogen fluoride and one mole of GB acid according to the following reaction:



Therefore, mere dissolution of GB in water is in itself a decontamination procedure (sometime termed "weathering" when dealing with field decontamination). Because hydrolysis of GB in distilled water is slow, it is not considered a good decon procedure per se.

However, if sodium hydroxide (NaOH) is added to a water/GB solution, a rapid bimolecular reaction is observed whereby hydroxide attacks the phosphorous center and subsequently releases a fluoride ion. Hydroxide is well known in the chemical literature not only as a strong base but as a excellent nucleophile. Thus the hydroxide anion attacks the phosphorous to form a transient intermediate, which then decomposes to produce fluoride anion. In the study of this reaction it is observed that increasing the hydroxide concentration increases the reaction rate; i.e., it is a bimolecular reaction which is dependent on the concentration of hydroxide. In practical terms, although only one hydroxide is involved in the initial attack at phosphorous, two molecules of hydroxide are consumed for every molecule of GB hydrolyzed because one of the products is itself an acid, isopropyl methylphosphonate. This second acid-base reaction is itself advantageous as it prevents the isopropyl methylphosphonate from re-reacting with fluoride to form GB. Thus the overall stoichiometry of sodium hydroxide with GB is represented by the following:



Note that the reaction produces one mole of fluoride ion, one mole of isopropyl methylphosphonate (GB acid anion), and consumes two moles of hydroxide.

The stoichiometry above demonstrates why NaOH is recommended as a decon reagent. NaOH is very soluble in both aqueous and aqueous alcoholic solutions, and is a potent nucleophile and base in almost all solutions. It is one of the first decon reagents to be used in the chemical-warfare arena and continues to enjoy popularity as a rapid, complete and inexpensive procedure. Note in the NMR data in the attached appendix that the sole product of the hydrolysis of GB in aqueous sodium hydroxide is clearly isopropyl methylphosphonate reaction (GB acid). Note also that the reaction is complete (>99.5%) in less than 10 minutes.

Why, if hydroxide is such an effective decon reagent, are numerous other solutions recommended to perform this transformation? The answer is that this NaOH is very corrosive. This solution is extremely damaging to many metal surfaces and is potentially quite damaging to skin, clothes, and other materials. Of particular concern is the well-known reaction between aqueous NaOH and aluminum metal. This reaction produces hydrogen gas in a very exothermic transformation and is extremely damaging to any component which contains aluminum. In several industrial processes this reaction between hydroxide and aluminum to form sodium aluminate (producing heat) is used commercially, and is often found in household drain cleaners such as "Drano." Therefore, the decontamination of equipment which contains reactive metals such as aluminum or magnesium requires alternative reagents to hydroxide in order to suppress this corrosive reaction. One of the most useful is the substitution of sodium carbonate for aqueous sodium hydroxide in the decontamination solution. At first glance this seems a major change in the decon procedure, but, in fact, sodium carbonate is well known to react with water to produce sodium bicarbonate and hydroxide according to the following reaction:

$$Na_2CO_3 + H_2O \rightarrow NaHCO_3 + NaOH$$

The advantage to this procedure is that it produces an alkaline aqueous solution (a solution defined as containing a greater concentration of OH^- ions than H^+ ions) when dissolved in water, and undergoes hydrolysis to yield sodium hydroxide which then may proceed to act as a potent reagent against GB. The major advantage to sodium carbonate is that although it releases hydroxide in the solution on a steady basis, the hydroxide is simultaneously consumed by another reaction (i.e., the reaction with GB to form isopropyl methylphosphonate and floride, reaction given above). ***Thus sodium***

carbonate, although not as powerful a reagent as pure sodium hydroxide, produces sodium hydroxide in solution and is chemically equivalent to hydroxide in its chemistry.

In the following table note the pH relationships between sodium hydroxide, sodium carbonate, and other similar bases. Note also that a 0.01 M solution of sodium carbonate has approximately the same pH as 0.001 M sodium hydroxide. When used as a decon solution, therefore, sodium carbonate is a mild source of hydroxide ion (the active nucleophile in solution) and thus is more advantageous as a decon reagent when aluminum and other reactive metals are exposed to the decon process.

Approximate pH Values for Various Concentrations of Selected Bases

Compound	1N	0.1N	0.01N	0.001N
Ammonia	11.8	11.3	10.8	10.3
Potassium Hydroxide	14.0	13.0	12.0	11.0
Sodium Carbonate	-	11.5	11.0	-
Sodium Hydroxide	14.05	13.07	12.12	11.13

In the attached appendix it can be seen that both GB and GD are hydrolyzed in aqueous sodium carbonate, aqueous sodium hydroxide, and alcoholic sodium hydroxide to form rapidly the same decontamination products. Kinetics on these systems suggest that both GB and GD have a half-life during hydrolysis under these conditions on the order of 5-10 seconds; only one to two minutes of reaction are needed to proceed through 10 half-lives of hydrolysis. Nuclear magnetic resonance (nmr) data show that after 5 minutes essentially no GB can be observed when this technique is used. Under these experimental conditions, were GB present in concentrations greater than 0.5%, it would be clearly observed in the phosphorous spectrum. Thus product analysis (nmr data attached) and kinetic data are consistent with the observation that both NaOH and Na_2CO_3 in aqueous solutions rapidly react with GB to form isopropyl methylphosphonate anion and nothing else. The usual recipe for using sodium carbonate recommends the solution remain in contact with agent for 30 minutes. If, at 5 minutes, 10 half-lives of reaction are completed, then in 30 minutes approximately 30 half-lives of reaction will have been completed. This allows a calculated theoretical concentration of GB in aqueous sodium carbonate at well below the ppt level (see discussion of kinetics below).

In many other decon solutions, hydroxide or a hydroxide-producing reagent is recommended. Note the use of amines in various decontamination formulations. Again, amines dissolved in water are well known to hydrolyze to form aqueous hydroxide by the following reaction:

$$R_3N + H_2O \rightarrow R_3NH^+ + HO^-$$

This reaction is most often encountered in the use of commercial cleaners which contain ammonia. Ammonia, an amine very soluble in water, dissolves to form ammonium hydroxide, NH_4OH. It can therefore be seen that aqueous ammonia solutions are a source of hydroxide ions as is sodium carbonate. Substituted amines, such as monoethanolamine, dissolve in water to form hydroxide ion even more efficiently than ammonia. Therefore, any decon solution which contains low molecular weight amines rapidly produces an aqueous alkaline solution (i.e., hydroxide in solution). All evidence to date indicates that hydroxide in contact with GA, GB or GD produces a very rapid reaction to form the acid salts of these agents.

A similar situation exists with the use of oxidizing reagents. The familiar household bleach "Clorox" is an aqueous solution of sodium hypochlorite, NaOCl. This material is a chlorine oxidant of very powerful reactivity. It is an excellent disinfectant and a useful oxidizing agent against a large number of organic compounds, especially those which contain sulfur. In household cleaning applications this aqueous solution of hypochlorite oxidixes such biological materials as bacteria and fungis into non-living states. It is also useful for oxidatively degrading stains of various types to smaller, more soluble fragments which then can be removed by detergents in the washing medium.

The use of hypochlorite in decontamination against mustard (HD) and VX is similarly related to the oxidation potential of the hypochlorite anion (^-OCl). The oxidation potential of this anion is such that that care must be taken in its use because of the heat generated as reactions proceed. In most applications, aqueous solutions are recommended to moderate the oxidation reaction and reduce the danger involved. Therefore any source of hypochlorite is a good decontaminant for oxidizable groups such as mustard and VX. Numerous recipes exist for the inclusion of reagents which produce hypochlorite ion in water. These include, for example, sodium hyplochlorite (5% "Clorox" strength), calcium hypochlorite (HTH or STB) as an aqueous solution or slurry, and the soft halogens such as fichlor and chloramine B, which produce hypochlorite upon reaction with water. Thus the formulation of an oxidative decontamination solution follows the same general orthodoxy as one observes with aqueous sodium hydroxide; i.e., the choice of the oxidant depends on the substrate to be deconned, but in all cases the reagent produces a controlled amount of hypochlorite which actually performs the decon reaction. In this context sodium hypochlorite, calcium hypochlorite, or the organic N-chloramine compounds can be considered as chemically equivalent because they react with water to release hypochlorite as the active ingredient.

Yet another reactive attribute of hypochlorite is exploited in many detoxification reactions. Hypochlorite falls into the category of alpha nucleophiles (see Section 4.1.4.2), powerful reagents similar to hydroxide in nucleophilic behavior (in contrast to oxidative behavior). Therefore the hypochlorite anion will react as a catalyst with G-agents to hydrolyze the material. The underlying reason why these types of anions are such powerful nucleophiles is still debated in the chemical fraternity; however, it has been demonstrated that they are very reactive against phosphorous compounds such as GB. An aqueous hypochlorite solution is a powerful decontaminant against G-agents through this hydrolytic mechanism as it is with mustard and VX through oxidation. This dual reactivity of hypochlorite has recently exploited by the German Army's fielding of the "C8 emulsion". This recipe is an aqueous organic emulsion which contains calcium hypochlorite as an active oxidant. It is extremely powerful in decontaminating mustard and other sulfur-containing compounds. CRDEC has demonstrated that this formula is also a powerful decontaminant against G-agents. In the product analysis of G-agents, the normal hydrolytic products are rapidly produced (i.e., isopropyl methylphosphonate from GB), as shown in the nmr data (Appendix attached).

The discussion demonstrates that although numerous decontamination recipes have been suggested for various procedures, all the reagents are very similar, if not identical, in their reactive behavior and can be considered chemically equivalent decontamination procedures on a mole per mole basis. Thus, for example, no major differences should be inferred when sodium carbonate is substituted for sodium hydroxide insofar as mechanism and products are concerned. Although this view has been assumed in many early studies, it is clearly documented at present through the use of nmr technology (Appendix attached).

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4 Historical Background

4.1 Decontamination Methods - Archives

4.1.1 INTRODUCTION

4.1.1.1 Purpose and Scope of Survey

The purpose of this survey was to identify all archival material reported for the decontamination and/or destruction of the listed CW agents.

The methods used for neutralizing an agent are strongly influenced by the amount of agent present and its environment. As there is no universal decontaminant for each agent, it is desirable to have at hand a listing of all of the reported techniques, which will serve not only for ordinary situations, but also as a starting point for developing procedures for extraordinary situations.

This literature survey covers the period 1918-1987. In it are included open literature publications, government reports and industrial contract reports.

4.1.1.2 Organization of the Archival Material

Whereas a large number of decontaminating systems or methods have been studied for the destruction of distilled mustard (HD), G-Agents (GA, GB, and GD), S-(2-diisopropylaminethyl)-O-ethyl methylphosphonothioate (VX), and Lewisite (L) they can conveniently be subdivided into several categories: 4.1.2. water; 4.1.3. strong bases; 4.1.4. complexing agents and nucleophiles (other than 2.); 4.1.5 oxidants; 4.1.6. photochemical methods, and 4.1.7. physical collection. In this review, each category will be considered in turn. Where reported, the following, if available, will be included for each reference: quantity of agent processed, percent destroyed, reaction kinetics and method of analysis.

Those analytical methods that have been included in standard operating procedures (SOP) will be considered in appreciable detail in Sections 4.1.8 and 4.2.5.

4.1.2 WATER

Both fresh water and sea water, although plentiful and inexpensive, are relatively ineffective agents for the destruction of CW agents; nevertheless they have been used to wash contaminated surfaces. (1) The solubility of HD in water is low[2], 1 g/L, and the hydrolysis rate constant is relatively low (0.13 min^{-1}) at ambient temperature. (2) Mustard is 99.3% hydrolyzed at 50°C in 100 minutes.[3] Increasing the temperature of the water,[4] or using steam,[5] causes volatilization of a portion of the HD.[6] Addition of detergents, such as the alkyl

sulfonates or quaternary ammonium compounds, increases the solubility of HD 8 to 20 times, but results in hydrolysis rates 3 to 20 times slower.[2] The same situation results with the use of detergent micelles.[7]

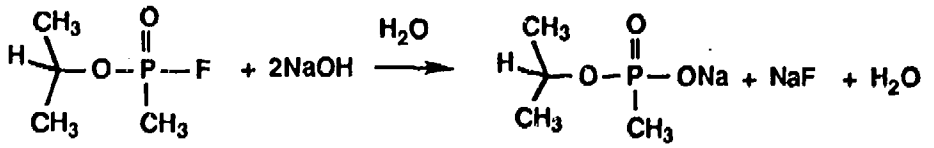
The organophosphate GB is completely miscible with water and its hydrolysis half-life in dilute solution is 75 hr at pH 7 and 25°C,[8] which is too slow from a practical standpoint for decontamination. Similarly, for VX, the values are, 30 gm/l of water (solubility), and 40 days at pH 7, respectively.[8]

Neat Lewisite (L) in water reacts rapidly to give lumps that are soluble only on prolonged stirring and are polymeric modifications of the oxide ClCH=CHAsO.[133] The aqueous solution of the oxide has vesicant properties.[134]

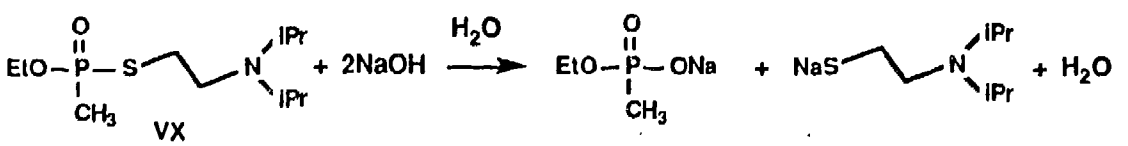
4.1.3 STRONG BASES

4.1.3.1 Aqueous Solution

Strong bases in aqueous solution may be defined as those giving a pH of approximately 11 or greater. Cleavage rates for GA, GB, GD and VX are proportional to the hydroxyl concentration (see discussion in 3.3.3), while for HD, rates in basic solution are comparable to those in water alone.[7,10,11] Unfortunately, the higher pH solutions are more corrosive to skin and to various materials. Hydrolysis of GB in strongly basic solution involves the equation:



The second order reaction rate is 30 $M^{-1} \cdot s^{-1}$ and the heat of reaction is -44.4 kcal/mole.[12] With 5% aqueous sodium hydroxide (pH > 14), the half life of GB is <0.8 sec. With respect to VX, the pertinent equation is:



VX

The half life of VX at pH 14 is 1.3 min[13], and the second order rate constant is $30 M^{-1} \cdot hr^{-1}$, but because of its relatively low solubility in water (above about 9°C), the reaction requires a considerably longer time unless an organic solvent such as 2-methoxyethanol is included. Therefore for VX (and HD),

actual rates will be slower, depending upon the rate of solution of agent, which will depend in turn upon the degree of mixing of the heterogeneous systems.

Lewisite reacts with aqueous sodium hydroxide as follows: [134,135]

$$ClCH{=}CHAsCl_2 + 6\ NaOH \rightarrow Na_3AsO_3 + 3\ NaCl + H_2C_2 + 3\ H_20$$

The isomeric (cis and trans) Lewisites react at different rates in 16 % aqueous sodium hydroxide, with one isomer giving almost complete acetylene evolution in 2 min and the other requiring approximately 1 hr.[136] Because of the relative insolubility of Lewisite or its oxide in aqueous solution,use of a cosolvent such as alcohol is recommended.

Many bases have been studied for decontamination. Whereas the use of 10% aqueous sodium hydroxide has been reported to be effective against HD,[14] later reports indicated the opposite.[15,16] In another report, Reichert[17] found that 125-gallon batches of HD could be effectively decontaminated with 125 gallons of water at 70°C, plus calcium oxide in excess, which raised the temperature to 100°C. The mixture was allowed to stand overnight. Analysis *via* thin layer chromatography (TLC) and gas-liquid chromatography (GLC)-mass spectrometry (MS) indicated complete hydrolysis to thiodiglycol and calcium chloride, plus some polysulfide residue that separated. The author also mentioned the use of aqueous sodium hydroxide and of ammonium hydroxide for hydrolysis of HD, but there was no indication that these bases had been used for large-scale decontamination.

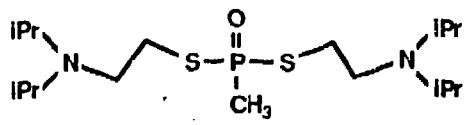
By contrast with HD, aqueous sodium hydroxide has been used as a standard method for the decontamination of bulk GB from munitions. The reaction yields sodium isopropyl methylphosphonate and sodium fluoride. It has been employed for demilitarization of the M55 rocket[18] and M139 and E139 bomblets,[19] among other applications. Once the GB has been hydrolyzed, the brine solution is dried prior to disposal. A voluminous literature[20-30] base has resulted for the testing for residual GB in the brine, in the stack emission, and in the dried salts. There are two standard methods for GB trace analysis, enzyme and GLC. Both require extraction of residual GB from the material of interest with a polychlorinated alkane. For the enzyme method,[23,24] which is more sensitive but less specific and subject to more interference, the extract is usually subjected to a preliminary TLC separation[31] followed by scraping-off of the spot, reaction with cholinesterase and by a pH, colorimetric or fluorometric measurement. The GLC procedure,[25,32-34] is less sensitive, but more specific (Section 8).

Because sodium hydroxide solutions produce hydrogen with the aluminum often accompanying the GB in munitions, less basic solutions have been investigated. One of these is aqueous sodium carbonate,[12,35] which is less corrosive for aluminum. The half life of GB in this solution was reported to be 8.5 seconds with a first order rate constant of 0.08 s^{-1} and a destruction efficiency of >99.9999%. The heat of reaction (ΔH) with 10% sodium carbonate

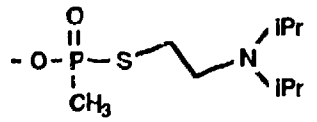
has been estimated to be -22 kcal/mole. This was shown to give a "safe" temperature rise of 2.58°C for an adiabatic process using 300% excess reagent (one pound of GB per seven gallons of 10% sodium carbonate.) Methods of analysis were essentially the same as those for hydroxide brines. Nmr analysis of spent 10% carbonate solutions indicate GA, GB, and GD destroyed to 99.5% in 5 minutes at room temperature (see Appendix).

While VX is more resistant to cleavage by bases than GA, GB, and GD, it has been decomposed with aqueous sodium hydroxide.[36] Decontamination of 12.5 gallons of VX by 150 gallons of 10% sodium hydroxide required 6 to 8 hours with air agitation at 25-30°C. This technique was recommended by Monsanto,[37] but the sulfur and nitrogen degradation products, including diisopropylaminoethanol are not commercially reusable.

Similar studies were reported by the Navy.[38] A total of 12.5 gallons of VX was decontaminated using 150 gallons of 10% aqueous sodium hydroxide (air agitated) in three stages (50 gallon addition, each stage). The solubility of VX was initially incomplete. The last two stages employed heated sodium hydroxide solutions. The time for "complete"decontamination was 6-8 hrs. The solubilization problem indicates that this method of decontamination will be unreliable unless the mixing process can be very adequately controlled. It must also be noted that if the reacting VX contains the "Bis impurity", the action of base will generate a refractory compound (see formula below) which undergoes further hydrolysis slowly. This refractory anticholinesterase is toxic by intravenous routes; the oral toxicity is considerably less. However, this material, in aqueous or alcoholic solution, is apparently not absorbed through the skin; no effects were found on application in water or alcohol to the backs of clipped rabbits.[47] The compound is crystalline when pure (mp = 138-140°C) and is infinitely soluble in water and ethyl alcohol; as such it is not a vapor hazard.

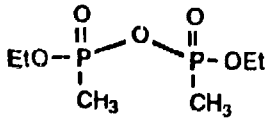


"bis"analog of VX



refractory anticholinesterase

In work done to support the Demil plan at the Tooele Army Depot[21] three decontamination procedures were evaluated for large scale destruction of VX. These included alcoholic caustic, calcium hypochlorite, and chlorine gas in acidic media (acid chloronolysis). It was suggested than in addition to the two analogues discussed above, the base reaction also produces the "pyro" compound, structure shown below) by reaction of the O-ethyl methyl phosphonic acid anion (initial hydrolysis product of VX) with VX.



This product also hydrolyzed on standing with aqueous base. This study reports that incomplete reaction of VX with either hydroxide or calcium hypochlorite produced solutions which gave weak toxicological results (by intravenous injection) but that excess reagent produced solutions clean of any major toxicological response (rabbits and mice). The conclusion of these studies indicated that acid chloronolysis was the solution method of choice for large scale destruction of VX in Demil procedures.

Methods of analysis for residual VX in the brines and in the dried salts are similar to those for GB and involve extraction of agent followed by GLC (phosphorus and sulfur flame filters), or TLC, with enzyme analysis.[8, 21,39]

Sodium hydroxide also appears to be the decontaminant of choice for L. Nmr analysis show that treatment of L with 10% sodium hydroxide solution immediately evolves gas (acetylene) and produces a solution with >99.5% L destruction (no carbon signals present) in 5 minutes (See Appendix).

A number of other strongly basic sodium salts have been suggested[40] as substitutes for sodium hydroxide or sodium carbonate in the decontamination, including trisodium phosphate or sodium silicate, but they do not seem to have been studied in any detail.

4.1.3.2 Partly Aqueous and Nonaqueous Solutions

The main advantage in working in these media is that the agent is usually more soluble and hence should be more readily available for nucleophilic attack, other factors being equal. Yet the fact that partially or completely nonaqueous solutions have lower dielectric constants than water may slow the reaction. Also, there are often problems of toxicity and corrosivity connected with organic solvents. In the following examples, it should be noted that these reagents are preferred for small-scale decontamination, such as on skin, cloth, metal, or other surfaces.

Sodium sulfide, 15% in a mixture of glycerol, ethanol and water, required 20 hours at an ambient temperature to destroy HD.[6]

Sodium hydroxide in methanol reacts too slowly with HD to be effective, yet in ethanol, the half life is 11 hours.[14] While VX, like HD, is more soluble in alcoholic base, problems of flammability have lessened that decontaminant's use.[13] An effective skin decontaminant for HD was described by Steyermark,[41] which consists of a quaternary ammonium hydroxide or alkali metal hydroxide, alkoxide, or phenoxide in mixtures of dimethyl sulfoxide and water or alcohol. A mixture of 70% methyl cellosolve and 30% of a 50% aqueous sodium hydroxide solution [42] gave complete destruction of HD in 2 hours (verified by GLC) to yield thiodiglycol and sodium chloride. This agent has a relatively large capacity for HD decontamination and compared very favorably with other HD decontaminants.

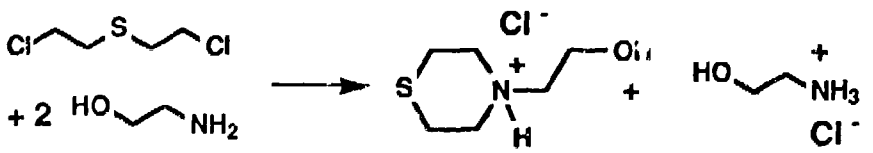
A number of multicomponent, strongly basic mixtures have been studied for the decontamination of HD, GA, GB, GD, L and VX. One of these is DS-2, patented by Jackson[43] as being effective, and relatively noncorrosive, and consisting of 70% diethylenetriamine, 28% 2-methoxyethanol and 2% sodium hydroxide. With this mixture, the half lives for HD, GB, and VX[44,45] were found to be 2.3 seconds, <30 seconds and <7 seconds, respectively, at ambient temperature. The products formed from HD included divinyl sulfide, which is somewhat toxic, but much less so than HD. In one report,[44] 25 cc of HD plus 1.33 quarts of DS-2 gave a 31% yield of divinyl sulfide in a very rapid reaction. Residual HD was determined via GLC. Other studies on DS-2 were made by Day[46] with HD, cyclohexyl methylphosphonofluoridate (GF) (GB analog) and VX on painted panels after standing overnight, and by Fielding[48] on various surfaces. Treatment was found to be effective for GB and HD, but somewhat less so for VX. The products from VX were tentatively identified as bis (2-diisopropylaminoethyl) disulfide,[44] presumably plus the O-ethyl methylphosphonic acid. For GB, the products are the same as those for hydrolysis in aqueous sodium hydroxide.

In work performed with DS-2 at Monsanto,[49] a thorough study was made on the function of the three components in the solution. It was postulated that the amine in DS-2 complexes with the sodium ion to give a superbase. Substitutes included crown ethers, polyethyleneimine and iminobis(propylamine). The 2-methoxyethanol in the standard DS-2 mixture was replaced as solvent by a variety of glymes in various formulations and the sodium hydroxide by lithium diethylamide. None of the substitute formulations was found to be markedly superior to DS-2, which gave 100% HD destruction at an ambient temperature in several minutes. Unfortunately, because DS-2 has a low sodium hydroxide content (2%), increased volumes (vs. HTH) must be used to obtain equivalent levels of decontamination. It is also corrosive to epoxy resins, neoprene, and wood.[40]

In studies made on the reaction of VX with ethanolamine, with hexyleneglycol added to give homogeneity, it was found that 70% of the VX remained intact after 2.5 hr at room temperature.[38]

Studies by the Navy[50] were made of benzyltrimethylammonium hydroxide in methanol as a decontaminant for small amounts of VX in the laboratory.

Monoethanolamine (MEA), an organic solvent, which is itself a relatively strong base, has been used for the decontamination of HD.[16] The use of MEA has a number of decided advantages[51,52] including: relatively high flash point, relatively non-toxic (TLV of 3 ppm), non-corrosive to metals, inexpensive, relatively stable, homogeneous reaction with HD, moderate heat of reaction and volume ratio of only 5:1 required. The reaction of HD and MEA is given by the equation:



The type of reaction represented by the above equation has received attention in the open literature,[38] but quantitative studies of products, kinetics and thermochemistry were not reported. A decided advantage of these systems is the absence of inorganic salts in the final disposition process. The products from HD, ie, N-(2-hydroxyethyl)thiomorpholine hydrochloride, monoethanolamine hydrochloride and small amounts of bis(hydroxyethylaminoethyl)sulfide, were incinerated at 900°C to give carbon dioxide, hydrogen chloride and various oxides of nitrogen and sulfur, which were collected in an 18% aqueous sodium hydroxide scrubber.[16]

The half life of this reaction was reported as being 11 min at 57°C and 40 min at 44°C.[38] The heat of reaction at 50°C was -40 kcal/mol. Above this temperature,the heat of reaction increased significantly and cooling was required. With a 5:1 v/v ratio of MEA to HD, the adiabatic temperature rises were from 50°C initial to 113°C final and from 65°C initial to 151°C final.

Studies have indicated that for chloroform solutions of various agents, reaction with MEA may yield a delayed violently exothermic reaction, especially in closed vessels. The hazard of a slowly appearing exotherm, which nevertheless results in a violent run-away reaction upon storage (reaction of MEA with solvent), is not an isolated instance in the history of stored materials resulting from disposal operations. Detailed methods and apparatus are being developed for safely eliminating the appearance of such unpleasant surprises.[38] Analysis of various systems are performed by computer-controlled adiabatic calorimetry with computer data-processing. Other approaches to the problem have been the previous use of differential thermal analysis (DTA) and differential scanning calorimetry (DSC), but the approach cited in the above reference develops much more complete information for analysis, if an actual problem exists. Detection of the problem should be adequately performed however, by DTA and/or DSC.

Several additional studies[51,52] led to the selection of MEA as a feasible decontaminant for HD. The compound has also been applied to the destruction of HD impregnated on charcoal[53]. When combined with 4-(N,N-dimethylamino)pyridine it has been employed for destruction of GB.[54]

4.1.3.3 Molten Salts

A novel technique for the destruction of chemical warfare agents involves the use of molten basic salts at elevated temperatures. In the method, first studied by Atomics International,[55-57] HD, GB, and VX in air, at feed rates of approximately 10 grams per minute, were passed through beds containing 90%

sodium carbonate and 10% sodium sulfate at 1000°C. The agents react according to the following equations:

$$HD + 2\ Na_2CO_3 + 7\ O_2 \rightarrow Na_2SO_4 + 2\ NaCl + 6\ CO_2 + 4\ H_2O$$

$$VX + 3\ Na_2CO_3 + 20.5\ O_2 \rightarrow Na_3PO_4 + Na_2SO_4 + NaNO3 + 14\ CO_2 + 13\ H_2O$$

$$GB + 2\ Na_2CO_3 + 6.5\ O_2 \rightarrow NaF + Na_3PO_4 + 6\ CO_2 + 5\ H_2O$$

The bench scale results were: HD off-gas <0.023 mg/m^3, particulate filter <30 ng, melt residue <30 ng/gm; GD off-gas <0.00049 ng/m^3, filter <50 ng, melt <100 ng/gm; VX off-gas gas <0.000085 mg/m^3, filters <1.5 ng, melt <3 mg/gm. These figures corresponded to agent destruction of ≥0.99999%.[58] Assay was *via* extraction followed by GLC-mass spectrometry for GB and HD or by enzyme analysis for VX.

The molten salt method has several problems, including the presence of phosphorus pentoxide particulates, requiring efficient particulate filters, and the presence of sodium chloride condensation in off-gas lines, requiring low temperatures for the molten salt.[59]

4.1.4 COMPLEXING AGENTS AND NUCLEOPHILES

4.1.4.1 Metallic Salts

These compounds customarily are employed in solutions closer to neutrality than are the bases of Section 3 above and are frequently much less corrosive. Various metal ions have been observed to increase the hydrolysis rates of GB in water,[60-65] especially those of copper (II), uranium (VI), zirconium (IV), thorium (IV), and molybdenum (VI). Only a few of these systems have actually been translated into useful decontamination procedures. In one,[45] VX and GB on sateen were treated with 0.1 M uranyl nitrate and 0.1 M thorium nitrate solutions; neither was too effective. In another report,[66] involving VX in solution, 95-98% was destroyed in 30 minutes with either zirconium (IV), nitrate or copper (II) nitrate and tetramethylethylenediamine. Also satisfactory was uranium (VI) dioxybis(5-sulfo-8-hydroxyquinoline), with half life for GB of 2.8 minutes at pH 10 and 24 minutes at pH 7.[67] Various metal salts complex with HD without actually decomposing it, including mercury (II) perchlorate.[68] These have been used to impregnate clothing, but are deactivated by perspiration.

Interest in decomposition of agents with metallic complexes has returned with investigations involving several promising compounds.[69]

4.1.4.2 Alpha Nucleophiles

While hydroxide anion readily attacks electrophiles such as HD, GA, GB, GD and VX, even more rapid reactions are given by various alpha nucleophiles, even though they are less basic. The enhanced reactivity is related to the

presence of an unshared electron pair on the atom next to the one bearing the negative charge, which decreases charge repulsion during interaction. In this group are anions of hydroperoxides, hypochlorites, oximes, and hydroxamic acids, with most literature references involving GB and VX.[60,70-74] While a number of these reactions have very favorable kinetics, as measured in the laboratory, only hypochlorites appear to have been used for large-scale decontamination and these properly fall under the heading of oxidants, considered below. In one report,[75] a mixture of sodium hypochlorite and sodium perborate was used for HD, but no rationale was given. In a hypothetical exercise,[13] a search was made for a hydroxamic acid that would decontaminate 400 gm of VX from a munition by dissolution in 1200 L of a 0.2 M aqueous solution of the hydroxamic acid at pH 7 to 9 to give a final agent concentration of 10^{-7} to 10^{-8} M after 1 hour. The sought-after acid was not found. A number of promising alpha nucleophiles have been synthesized by Reiner, which react rapidly with diisopropyl phosphonofluoridate (DFP, a G-agent simulant) including a-oxominovaleronitrile, ethylenediaminetetracetohydroxamic acid, amylose oxime, and pentafluoro-benzaldoxime.[76]

Besides alpha nucleophiles, bidentate nucleophiles such as pyrocatechol and pyrogallol anions[77-81] were found to hydrolyze organophosphates rapidly. Here, too, promising results in the laboratory have not been turned into practical systems.

Sodium thiosulfate reacts rapidly with HD,[82] but neither this reaction, nor one involving hydrolysis of GB at pH 7.6 in the presence of pyridinium bases,[83] has been applied to bulk quantities of agents.

4.1.4.3 Micellar Nucleophiles

In this group are oximes containing a large aliphatic moiety, which tends to concentrate on the surface of solution, where more favorable concentration effects should enhance organophosphate hydrolysis. As an example, the half life or VX in a pH 9.3 solution containing dodecylpyridinium-3-aldoxime iodide (10^{-3} M) was 40 seconds.[84]

4.1.5 OXIDANTS

4.1.5.1 Halogen

4.1.5.1.1 Calcium Hypochlorite

Of the agents under consideration in this report, two, HD and VX, contain sulfur moieties that are readily subject to oxidation. One of the first substances[6] used for the destruction of HD was "bleach", which is normally found in three forms: a 5% aqueous sodium hypochlorite solution (Clorox, Purex, etc.), chlorinated lime (a solid with the approximate formula CaClOCl) and calcium hypochlorite (HTH, with the formula $Ca(OCl)_2$). The last named, having the

highest percentage of available chlorine, is the form most often used for current decontamination.

The reaction of calcium hypochlorite with HD has been conducted in a variety of media.[14] With solid reagent, the reaction may be violently exothermic.[85] With hypochlorite in an aqueous slurry, the reaction is more easily controlled. This mixture has been recommended for the detoxification of buildings, ground, and other large-surface areas.[86-89]

While the reaction varies with the proportion of reactants and temperature, a proposed equation[15] for the maximum consumption of bleach is:

$$(ClCH_2CH_2)_2S + 14\ OCl^- \rightarrow SO_4^= + 16\ Cl^- + 4\ CO_2 + 4\ H^+ + 2\ H_2O$$

With a deficiency of hypochlorite, the sulfoxide and/or the sulfone of mustard may be produced.[90] Nmr analysis of the oxidation with excess bleach show conversion into numerous (estimated 20) products in greater than 99.5%, none of which were mustard (See Appendix). Toxicological test on mustard decontaminated with bleach show no toxic effects.

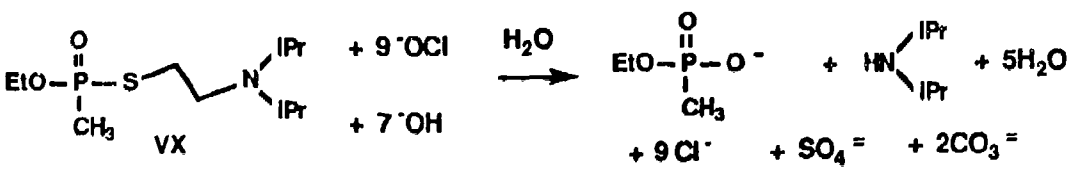
As HD is relatively insoluble in water, the reaction with aqueous calcium hypochlorite is a heterogeneous one and rates of decontamination have not been studied.[15] Nevertheless, several kinetic investigations have been made in dilute homogeneous solutions at various pH values.[91,92] In actual decontamination of HD with calcium hypochlorite,[93] scaled-down amounts, corresponding to ratios of 11.7 lb.of HD to 100 lb of HTH in 108 gal. of water were stored for several days at an ambient temperature, treated with sodium thiosulfate to remove excess hypochlorite and extracted with hexane. The extracts were submitted to GLC with sensitivity of 1 ppm of HD in hexane and results indicated essentially complete decontamination. The equation given for the reaction was:

$$(ClCH_2CH_2)_2S + 7\ Ca(OCl)_2 + 2\ Ca(OH)_2 \rightarrow CaSO_4 + 8\ CaCl_2 + 4\ CO_2 + 6\ H_2O$$

A bleach slurry was found to give complete decontamination of HD on Navy landing craft.[1] The agent HD is determined in bleach solution[33] *via* extraction and subsequent GLC (Section 8).

In order to speed up reaction, suspensions have been made of bleaching powder in organic solvents. One such solvent was carbon tetrachloride,[15] which was found to be superior to aqueous bleach paste, but was still slow because of the heterogenous nature of the reaction. Another example involved the use of 8% calcium hypochlorite in a mixture of 76% water and 15% chlorinated hydrocarbon, with 1% alkylbenzenesulfonate emulsifier.[94] Reaction occurred at the phase interface and theoretically, the system could be improved *via* inclusion of a phase transfer catalyst.[13] More efficient are the organic chlorinating agents discussed below:

Calcium hypochlorite has also been applied to the decontamination of VX, the reaction being given by the equation:[95]



The reaction is rapid, with a half life of 1.5 minutes at pH 10. It has been used for the demilitarization of VX in the CAMDS Project at Tooele Army Depot,[21] but it was considered to be less effective than is acid chlorinolysis. The major determinant of the transformation is that the pH is critical and toxic solutions can be formed if the pH drops to a value below 11 (see discussion above).

The reaction is highly exothermic[38], with an experimentally determined value of -675 kcal/mol and a first order rate constant of 0.01 s^{-1}. The rise in temperature can be calculated from the equation:

rise in°C = (moles VX)(175)/gal.10 % HTH

Initial results with extraction of trace amounts of VX from hypochlorite gave poor recoveries[96,97]. The current analytical procedure[33] is much more satisfactory (Section 8).

The reaction of L with hypochlorite has been studied, but because of the relatively slow kinetics of oxidation, it offers no advantage over aqueous sodium hydroxide.[137]

Self-destructing HTH solutions to limit corrosion have been prepared, with half lives of approximately 100 seconds. They are named ASH and SLASH,[98] contain citrate to remove excess active chlorine and have been used for biological agents.

4.1.5.1.2 Sodium Dichloroisocyanurate

Similar in action to HTH is sodium dichloroisocyanurate monohydrate (Fichlor, CDB-03), which possesses considerable aqueous stability and solubility (1 M/L) and has been used for laboratory-scale decontamination of VX.[99] The compound was reported for destruction of HD, GD, and VX on paint surfaces[100] and the test results were compared with those for other decontaminating agents. As with sodium hypochlorite, the stability of sodium dichloroisocyanurate in aqueous solution is pH dependent.[101] Because of its favorable characteristics relative to calcium hypochlorite, there is a current interest in Fichlor.[102]

4.1.5.1.3 Chloramine B, Chloramine T and NBO

Two other water-soluble active chlorine compounds of interest are Chloramines B and T. As compared to HTH, they have the advantage of greater stability[88,89] and less corrosiveness when applied to skin, but being more expensive, are not recommended for large-scale operations. Theoretical studies have been made on N-chlorinated compounds by Higuchi and coworkers.[103] In general, the weaker is the acidity of the NH base, the more stable the N-chloro compound. They react readily with tertiary amines and a number of them have been suggested as decontaminants for VX.[13] The products of reaction of chloramine B with HD include bis(2-chloroethyl) sulfoxide and the sulfilimine $C_6H_5SO_2N{=}S(CH_2CH_2Cl)_2$.[104,105] The proportion of the former increases with increasing water content.

An aqueous mixture containing 3-bromo-4,4-dimethyl-2-oxazolidinone (NBO) and cetyltrimethylammonium chloride in a bicarbonate/carbonate buffer has been studied for the decomposition of HD and VX as well as GD.[106] The solubility of NBO in the mixture is 0.14 M at 19°C. A 0.01 M NBO solution containing 0.0034 M HD gave < 1% HD (GLC) at 10 min (half life of 0.2 min). For VX and the reagent,at a 1:10 mole ratio,the half life of the VX was 0.2 min. Studies with GD (and by analogy GB)indicated that both hydrolysis and attack by reagent were occurring,with an agent half life of 0.5 min at a 1:1 mole ratio. Unfortunately,stability problems in solution have prevented greater use of this decontaminant.

4.1.5.1.4 Dichloramine B, Dichloramine T, DANC, and Other Water-Insoluble Active Chlorine Compounds.

This group of compounds is soluble in many of the solvents in which HD and VX are soluble. However, they are unstable in varying degrees to sunlight and to moisture.[107] The dichloramines have been applied in carbon tetrachloride solution,[108] in salves,[108] with inert solids such as kieselguhr or talc, or with alkali or alkaline earth carbonates or bicarbonates.[109]

Other examples for this group include N-chlorosaccharin,[13] N-chloro-succinimide,[13] N-chloracetamide,[110] N-chlorophthalimide,[110] bis (2,4,6-trichlorophenyl)dichloreurea,[111] and N-(2,3,6-trichlorophenyl) N-chlorobenzamide.[107]

Various other active chlorine compounds have been investigated.[112,113]

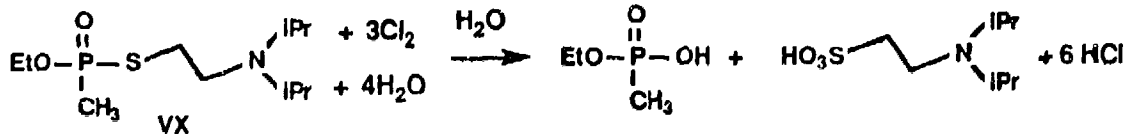
With N-chlorosaccharin, it was predicted[13] that at pH 8.6 in aqueous solution the half life of VX would be 10^{-4} seconds, but low water solubility among other factors, prevented application of the compound.

One formulation that was used extensively in the past was DANC,[14,15] a 7% solution of 1,1-methylenebis(3-chloro-5,5-dimethylhydantoin)(S-210) in tetrachloroethane. Other formulations[15] include: S-210 10.3%, tetrachloroethane 67.3%, barium hydroxide octahydrate 2.8%, Aristowax 1.6%,

and S-210 1%, tetrachloroethane 2.9%, Spar 201 4%, water 7%, remainder oil. In aqueous solution, S-210 reacts with HD to give a sulfilimine derivative.[95] Because of the high toxicity of tetrachloroethane and its corrosive effect on painted surfaces and rubber, DANC has become obsolete.

4.1.5.1.5 Chlorine and Chlorine Dioxide

Chlorine has been used as a large-scale decontaminant for VX, based upon earlier laboratory studies (half life 1.2 minutes at pH4).[114] In the actual procedure carried out at the Tooele Army Depot,[115] 100-pound batches of VX from munitions are dissolved in 1.5 N hydrochloric acid (1:3 v/v) and chlorine is added to a green color. Reaction is rapid and strongly exothermic. Samples are quenched with sodium hydroxide or sodium carbonate and extracted with dichloromethane. Residual agent (3 µg/L) is determined via fluorimetry,[116] TLC and an enzyme assay[23] or GLC.[117] The destruction efficiency was determined to be 99.999999%. The reaction is:



Also found among the products was dicyclohexylurea from the dicyclohexylcarbodiimide stabilizer in the VX. The solution from the chlorinolysis was converted to drum-dried salts.[118]

Chlorine dioxide reacts with VX to give carbon dioxide, carbonyl sulfide, sulfate ion, phosphonic acid and diisopropylamine.[119] As with chlorine, kinetics are very favorable, but the explosive nature of the gas would tend to preclude large-scale work.

4.1.5.2 Other Oxidants

An early oxidant used for the destruction of HD was potassium permanganate in acetone,[6] for cleaning of metallic instruments. Neutral permanganate was reported to completely detoxify (enzyme-assay) VX at a 20:1 molar ratio.[119] Among the products were ethyl methylphosphonic acid, N,N-diisopropylformamide, sulfate ion and gelatinous manganese dioxide, which along with unreacted permanganate, presented disposal problems. When VX was reacted with permanganate in highly basic solution,[13] the products formed indicated that hydrolysis predominated over oxidation.

Potassium peroxydisulfate, in combination with a silver ion catalyst, has been suggested for the decomposition of VX,[13] but no experimental work seems to have been done.

Oxidation of mustard with concentrated nitric acid cleanly produces >99.55 mustard sulfoxide (See Appendix).

Peracetic acid gave unimpressive results with GB and VX on sateen swatches.[45]

Various free radical systems were studied for oxidation of HD, GB, and VX, but it was concluded that the approach showed little promise.[120,121]

Novel oxidations of various agents with organic iodo compounds have been reported.[122,123,124]

4.1.6 PHOTOCHEMICAL METHODS

Little work has been done using this approach. Both HD and VX contain sulfur atoms that are subject to oxidation. One system that has been proposed for VX involves cold aerial photooxidation with photosensitizers such as Rose Bengal.[13,125] The decays in GB and VX clouds as they travel downwind, due to photolysis, hydrolysis and oxidation, have been reported.[126]

4.1.7 PHYSICAL COLLECTION

Physical collection removes the agent from one location to another without actually destroying it. It is primarily of value for the decontamination of surfaces or the removal of agent from water. Washing surfaces with water, water with detergent, or ethanol, has been used for decontamination.[127]

An early method of physical collection involved adsorption of various toxic chemicals.[128] A more recent technique is that of reverse osmosis[129] for removal of GB and VX from water with cellulose acetate and polyamide membranes. Agent concentrations were significantly reduced, but not always to a permissible level.

Ion exchange resins have been employed to remove small amounts of VX from hypochlorite brines,[96] but this was an analytical technique rather than a method of decontamination. Amberlyte-15 resin (Rohm and Haas Corporation) was studied for the removal of GB, VX, and HD from air.[135] Basic resins absorbed GB and possible hydrolytic products, then catalyzed the hydrolysis of GB.[131]

A review of ion-exchange methods reported for decontamination,with proposals for future work,was published in 1983. Among the recommendations were ultra-fine resin-zeolite slurries as general-purpose noncorrosive surface decontaminants and mixed bed cation-anion exchangers for potable water decontamination.[132]

Aqueous charcoal slurries (23-28%) in water, plus corrosion inhibitors and antifreeze compounds, have been mentioned for decontamination.[40]

4.1.8. ANALYTICAL PROCEDURES FOR STANDARD DECONTAMINATION METHODS

The standard method for the determination of residual GB in 18% sodium hydroxide solution requires initial adjustment of the pH to 5.0 with dilute sulfuric acid,followed by extraction with chloroform,preconcentration of the extract using Chromosorb 106 and GLC analysis.The column type is DB-210 bonded-phase fused silica capillary,15 m long by 0.53 mm ID, with a 1.0 um coated thickness of the stationary phase.The detector mode is phosphorus specific and the detection limit is 6.3 ppb.[33] An essentially identical procedure is used for the determination of GB in scrubber solutions and in sodium carbonate brines with detection limits of 4.8 ppb and 6.3 ppb respectively. Gas chromatographic analysis of GB also has been reported using a DB5 megabore column 30 m by 1.5 μ (J & W Scientific) with a detection limit of 0.05μg/mL of injected sample.[34]

A colorimetric technique for GB using o-dianisidine and perborate has a reported detection limit of 0.5 μg/ml,while an autoanalyzer procedure utilizing acetylcholineesterase and 5,5-dithiobis-2-nitrobenzoic acid claims a value of 0.25 ng/mL for the agent in an air stream.[34]

The agent HD is determined in bleach solution according to the following procedure.[33] Excess bleach is neutralized with aqueous sodium arsenite, the end point being determined bipotentiometrically.Extraction is made with chloroform followed by preconcentration on Tenax-GC. The gas chromatography column is DB-210 bonded-phase,fused silica capillary, 15m long by 0.53mm ID, with a 1.0μm coating thickness of the stationary phase. The detector is sulfur specific, with a detection limit of 39.4 ppb.

The analysis of residual VX in hypochlorite requires an extraction prior to GLC. Initially, *n*-hexane was the extractant, with a detection limit of 0.6 μg/mL, but recovery was poor.[96,97] The current method involves extraction with chloroform after a preliminary removal of excess hypochlorite with arsenite (see HD analysis above) and increase of pH to 10.0. Preconcentration requires adsorption on Chromosorb 106 and conversion to a fluoro compound similar to GB by reaction with silver fluoride.The chromatographic column and detector are the same as those for GB and the detection limit is 11.4 ppb.[33] A DB 608 column has been suggested for analysis of VX[34] as well as a DB-5 megabore column (30m, 1.5μ, J&W Scientific) with a detection limit of 1μg/mL for the latter.

Several proposals have been made for the analysis of L in trace amounts. In one (E.W.Sarver, Unpublished Results, 1974), the agent is reacted with 1,2-ethanedithiol to give 2-(β-chlorovinyl)-1,3-dithioarsenole, which is submitted to GLC.Another proposal involves a preliminary separation *via* high performance liquid chromatography coupled with amperometric assay, with an estimated detection limit of 1 ppm.[138] Preliminary studies have been made (S.Hallcwell,Unpublished Results,1976) of the titration of Lewisite with sodium 1,2-propanedithiol-3-sulfonate and a sulfide electrode.

4.1.9 CONCLUSION

Extensive decontamination experience and comprehensive data bases reviewed above have underwritten huge demilitarization projects in the past including GB-filled M55 rockets, and M139 and E139 bomblets, as well as research level decontamination protocol's utilized here at CRDEC and in other U.S. Army and free world instillations. An extensive evaluation of various of the above decontamination methods with respect to reliability, simplicity, safety and cost was made by the Jet Propulsion Laboratory.[139] In all cases, decontamination and disposal projects for agent-filled munitions were executed safety, without untoward incident, and in total compliance with every prevailing environmental and human safety stricture and concern at the time of the operation. As can be seen from the analytical data reviewed the thermodynamics, kinetics and product analysis documentation is extensive and has improved in recent times by advances in analytical hardware, as exemplified by the nmr data reported in the Appendix of this document. These modern analytical tools have by-in-large confirmed the archival data. These and other facts enumerated in detail in this document provide ample evidence that existing decontamination protocols and procedures are safe, scientific, and result in the total destruction and detoxification of chemical agents.

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4.2 Decontamination Protocols - Current

4.2.1. Background - Hazard Evaluation of Decontaminated Liquid Waste at CRDEC

In January 1986 the State of Maryland passed a regulation listing residues of certain decontaminated chemical surety material (CSM) as hazardous waste. Chemical Research, Development and Engineering Center (CRDEC) then initiated a delisting request for these residues, in that they do not meet the criteria for hazardous waste. CRDEC has tasked Research Directorate to provide both analytical and toxicological data that will support this delisting process.

To answer the questions posed to CRDEC by the State of Maryland in their September 1987 letter, the following criteria must be met:

a. CRDEC must provide a detailed description of the actual decontamination procedures used on the laboratory materials. This must include a step-by-step outline of the decontamination process, and must identify the decontamination agent used on a given CSM, the theory of these chemical reaction(s), the concentration of the decontaminating agent used, the amount of time the reaction is allowed to proceed, and note the parameters that influence the degree to which the reaction goes to completion.

b. CRDEC must describe the procedures used to assure that the solutions on which toxicological tests are performed are equivalent to the solutions resulting from the actual decontamination procedures.

c. Finally, CRDEC must describe the protocol for the toxicological testing so that the State of Maryland can determine whether it follows generally accepted practices.

In line with the above questions, this protocol describing in detail preparation of solutions (part b) used by Toxicology Division to verify the decontamination of the CSM in question (Question c above). These were taken from actual decon procedures used at CRDEC. For these tests a decon solution was prepared and divided into two portions. The first portion was analyzed to ensure destruction of agent and analysis of products. The second portion was subjected to toxicological testing. Thus data from these experiments will directly answer the question posed by the State of Maryland as to " procedures used to assure that the solutions on which toxicological tests are performed are equivalent to the solutions resulting from the actual decontamination procedures." The results of this testing schedule is given in Section 4.3.4 below.

The standard decontamination solutions used at CRDEC were chosen for completeness of the decon procedure. The theory of the decon reactions are covered in Sections 2 and 3 and the exact steps involved in making the decon solutions are covered in the General Protocol below. The portion of the solutions used for toxicological testing were adjusted after the decon procedure to ensure good laboratory practice requirements in the toxicological protocol outlined in 4.3.2. Toxicology Division determined by oral and inhalation route in rats, and by the dermal route in rabbits, that tested CSM were detoxified to a level less than a Class "B" poison using currently approved test procedures as spelled out in CFR 49 Department of Transportation (DOT) tests. The toxicity criteria for a class "B" poison are the same as the State of Maryland's criteria for hazardous waste.

4.2.2. Hypothesis.

Chemical decontamination of CSM, followed by neutralization and subsequent oral, dermal and inhalation toxicity tests, will show that the original

CSM have been decontaminated/deactivated in line with the State's letter to require that "residues no longer contain materials for which it was listed.

4.2.3. Approach.

The following agent/decon systems are routinely used for decontamination of listed agents in research quantities. Nmr evidence is given in Appendix as to the extent of reaction. Note in all cases that the nmr technique is accurate only to 99.5% destruction. Other analytical procedures must be utilized for more accurate quantitation.

	Agent	Recomended Decon Solution
1.	GA, GB, GD, L	10% NaOH
2.	GA, GB, GD, L	10% Na_2CO_3
3.	GA, GB, GD,	10% Alcoholic NaOH
4.	VX	10% $Ca(OCl)_2$ (HTH) plus 10% alcoholic NaOH
5.	GA, GB, GD, VX	10% $Ca(OCl)_2$ (HTH)
6.	GA, GB, GD, HD, L	5.25% NaOCl
7.	HD	Concentrated HNO_3
8.	GB	Monoethanolamine, Neat and 25% aqueous

Agent/Decon systems are selected on the basis of wide use in Research Directorate and Research, Development, & Engineering Support Division. Of the nerve agents, VX is the most difficult to decon by virtue of its reduced reactivity and solubility relative to the G agents; thus, both a VX/10% $Ca(OCl)_2$ (HTH) plus 10% alcoholic NaOH (standard solution 4 above) and a VX/10% $Ca(OCl)_2$ (HTH) (standard solution 5 above) system are reported. The decon of GB and GD are similar with the exception that GB it is more soluble and hydrolyzes more rapidly then GD. Extensive toxicological data for GB already exists. In order to minimize precipitation during HD decontamination in research quantities, NaOCl is used as the source of chlorine instead of $Ca(OCl)_2$.

4.2.4. Step-by-Step Outline of the Decontamination Process. The following are examples of the general protocol for listed compounds as decontaminated using accepted protocols at CRDEC.

4.2.4.1. Materials

4.2.4.1.1 10% NaOH Solution: A stock 10wt % NaOH/water solution will be prepared using distilled water and dry sodium hydroxide pellets of flakes in a ratio of 100g NaOH to 900g water. NaOH should appear dry and not stick together. The mixture should be stirred until all NaOH has dissolved.

4.2.4.1.2 10% Alcoholic NaOH Solution: 100 mL of denatured ethyl alcohol (95%) is added to each 900 mL of the NaOH solution described in paragraph 4.1.4.1.1.

4.2.4.1.3 10% Na_2CO_3 Solution: A stock 10wt % Na_2CO_3/water solution will be prepared using distilled water and dry sodium carbonate powder in a ratio of 100g Na_2CO_3 to 900g water. The Na_2CO_3 should appear dry and not stick together. The mixture should be stirred until all Na_2CO_3 has dissolved.

4.2.4.1.4 10% HTH Solution: A stock 10 wt % $Ca(OCl)_2$/water solution will be prepared using tap water in a ratio of 100g HTH to 900g water. The HTH should appear dry and not stick together. The mixture should be stirred until all the HTH has dissolved. Dry HTH typically contains 55% $Ca(OCl)_2$. Appropriate additions of dry HTH must be made if the HTH contains less than 55% $Ca(OCl)_2$. The amount of dry HTH may be computed by:

[55/percent purity of $Ca(OCl)_2$] X 100g = g of HTH in 900 mL water

In no instance will dry HTH with less than 30% $Ca(OCl)_2$ will be used.

4.2.4.1.5 Alcoholic HTH Solution: 100 mL of denatured ethyl alcohol (95%) is added to each 900 mL of the HTH solution described in paragraph 4.2.4.1.4.

4.2.4.1.6 5.25% NaOCl Solution: Commercial grade 5.25% strength aqueous NaOCl is used. The material must be certified, by analysis, to contain > 4.00% active chlorine before use.

4.2.4.1.7 Concentrated HNO_3 Solution: Commercial concentrated (65-70%) aqueous nitric acid is used undiluted.

4.2.4.1.8 Monoethanolamine, Neat: Material from supplier (> 95%, such as Aldrich) will be use directly without dilution with water.

4.2.4.1.9 25% Monoethanolamine Solution: A stock 25wt % MEA/water solution will be prepared using distilled water and neat liquid MEA in a ratio of 250g MEA to 750g water. The mixture should be stirred until all MEA has dissolved.

4.2.4.2. GENERAL INSTRUCTIONS FOR DECONTAMINATION

4.2.4.2.1 Decon procedures will be conducted at an ambient temperature between 20 and 26°C. Solutions will be allowed to come to ambient temperature before initiation of the decontamination protocol. Temperature will be recorded.

4.2.4.2.2 Agitation of agent/decontaminant mixture must be maintained for a minimum of one hour.

4.2.4.2.3 Agent must be added to the decontaminant (NOT DECONTAMINANT TO THE AGENT).

4.2.4.2.4 Reaction vessel must be large enough and open enough to withstand substantial exothermic reactions.

4.2.4.2.5 The concentrations of NaOH, Na_2CO_3 or $Ca(OCl)_2$ solutions represent the minimum to be used.

4.2.4.3. PROTOCOL FOR DECONTAMINATION OF GA

4.2.4.3.1 GA is decontaminated with the 10% NaOH solution (4.2.4.1.1). GA is soluble at 7 parts GA per 100 parts water.

4.2.4.3.2 A minimum of 55 grams of decon solution is required for each gram of GA. This ratio ensures that there is at least 22 moles of base for each mole of GA.

4.2.4.3.3 Decontaminant/agent solution is allowed to agitate for a minimum of one hour followed by reaction period of 23 hours with total decontamination assured after the solution has reacted for 24 hours. Agitation is not necessary following the first hour of the entire 24 hours.

4.2.4.3.4 At the end of 24 hours, the resulting solution should be titrated to a pH between 10 and 12.

4.2.4.3.5. After completion of the 24 hour reaction period, the decontamination solution must be treated with excess 5.25% NaOCl solution (4.2.4.1.7, commercial bleach, at least 2.5 mole OCl^- /mole GA) to destroy the CN^- formed during hydrolysis. For example, 20 g of GA is reacted with 1100 g 10% NaOH solution, then reacted with 600g 5.25% NaOCl (stoichiometric amount at 2.5 mole OCl^- /mole GA = 525g of 5.25% NaOCl). This solution is allowed to react for two hours to ensure destruction of cyanide. Before transfer to sump test for presence of active chlorine by use of acidic potassium iodide solution give free iodine color. If negative, add additional 5.25% NaOCl solution, wait for two hours, then test again for active chlorine. Continue procedure until positive chlorine is given by solution.

4.2.4.3.6. Alternate solutions for the decontamination of GA. It is permitted to substitute 10% Na_2CO_3 (4.2.4.1.3) for the 10% NaOH solution above. Continue with same ratios and the same time stipulations.

4.2.4.4. PROTOCOL FOR DECONTAMINATION OF GB

4.2.4.4.1 GB is decontaminated with the 10% NaOH solution (4.2.4.1.1). GB is miscible with water.

4.2.4.4.2 A minimum of 55 grams of decon solution is required for each gram of GB. This ratio ensures that there is at least 22 moles of base for each mole of GB.

4.2.4.4.3 Decontaminant/agent solution is allowed to agitate for a minimum of one hour. Agitation is not necessary following the first hour.

4.2.4.4.4 At the end of one hour, the resulting solution should be titrated to a pH greater than 11.5.

4.2.4.4.5. Alternate solutions for the decontamination of GB. It is permitted to substitute 10% Na_2CO_3 (4.2.4.1.3) for the 10% NaOH solution above. Continue with same ratios but increase the time of reaction from one to three (3) hours.

4.2.4.4.6 Alternate solutions for the decontamination of GB. It is permitted to substitute 5.25% NaOCl (4.2.4.1.6) for the 10% NaOH solution above. Continue with same ratios and the same time stipulations.

4.2.4.4.7 Alternate solutions for the decontamination of GB. It is permitted to substitute 25% MEA (4.2.4.1.9) for the 10% NaOH solution above. Continue with same ratios and the same time stipulations.

4.2.4.5. PROTOCOL FOR DECONTAMINATION OF GD

4.2.4.5.1 GB is decontaminated with the 10% NaOH solution (4.2.4.1.1). GD is miscible with water.

4.2.4.5.2 A minimum of 55 grams of decon solution is required for each gram of GD. This ratio ensures that there is at least 22 moles of base for each mole of GD.

4.2.4.5.3 Decontaminant/agent solution is allowed to agitate for a minimum of one hour. Agitation is not necessary following the first hour.

4.2.4.5.4 At the end of one hour, the resulting solution should be titrated to a pH greater than 11.5.

4.2.4.5.5. Alternate solutions for the decontamination of GD. It is permitted to substitute 10% Na_2CO_3 (4.2.4.1.3) for the 10% NaOH solution above. Continue with same ratios but increase the time of reaction from one to three (3) hours.

4.2.4.5.6 Alternate solutions for the decontamination of GD. It is permitted to substitute 5.25% NaOCl (4.2.4.1.6) for the 10% NaOH solution above. Continue with same ratios and the same time stipulations.

4.2.4.6. PROTOCOL FOR DECONTAMINATION OF VX

4.2.4.6.1 Procedure for decontaminating up to 50g of VX.

4.2.4.6.1.1 VX is decontaminated with the 10% HTH solution (4.2.4.1.4).

4.2.4.6.1.2 The minimum decontaminating solution to agent ratio is 8.25 moles of Ca(OCl)2 for each mole of VX. For the 10% HTH solution, 80 grams of decon solution is required for each gram of VX.

4.2.4.6.1.3 Solution is agitated or stirred for a minimum of one hour. If phasing of agent/decontaminant persists after 5 minutes, an amount of denatured ethyl alcohol equal to 10% (weight) of the total agent/decontaminant solution may be added to assist miscibility.

4.2.4.6.1.4 Upon completion of a minimum one hour agitation, the resulting solution is titrated to a pH between 10 and 12.

4.2.4.6.2 Decontamination of VX in excess of 50 grams.

4.2.4.6.2.1 A 10% alcohol HTH solution (4.2.4.1.5) is used to decontaminate 50g or more of VX.

4.2.4.6.2.2 Fourteen grams of alcoholic HTH solution is used for each gram of VX.

4.2.4.6.2.3 Solution is allowed to agitate for a minimum of one hour.

4.2.4.6.2.4 Upon completion of a minimum of one hour agitation, 10% NaOH solution is added to the resulting solution in a quantity equal to that necessary to assure that a pH of 12.5 is maintained for a period of not less than 24 hours.

4.2.4.7. PROTOCOL FOR DECONTAMINATION OF L (LEWISITE)

4.2.4.7.1 L is decontaminated with the 10% Alcoholic NaOH solution (4.2.4.1.2). L is poorly soluble in water.

4.2.4.7.2 A minimum of 200 g of decon solution is required for each gram of L. This ratio ensures that there is at least 78 moles of base for each mole of L.

4.2.4.7.3 Decontaminant/agent solution is allowed to agitate for a minimum of one hour. Agitation is not necessary following the first hour.

4.2.4.7.4 At the end of one hour, the resulting solution should be titrated to a pH greater than 11.5.

4.2.4.7.5. Alternate solutions for the decontamination of L. It is permitted to substitute 10% alcoholic Na_2CO_3 (solution 4.2.4.1.3 made up with 10% alcohol) for the 10% alcoholic NaOH solution above. Continue with same ratios but increase the time of reaction from one to three (3) hours.

4.2.4.7.6 Alternate solutions for the decontamination of L. It is permitted to substitute 5.25% NaOCl (4.2.4.1.6) for the 10% alcoholic NaOH solution above. Continue with same ratios and the same time stipulations.

4.2.4.8. PROTOCOL FOR DECONTAMINATION OF HD

4.2.4.8.1 HD is decontaminated with the 5.25% NaOCl solution (4.2.4.1.6). HD is poorly soluble in water.

4.2.4.8.2 A minimum of 65 grams of decon solution is required for each gram of HD.

4.2.4.8.3 Decontaminant/agent solution is allowed to agitate for a minimum of one hour. Agitation is not necessary following the first hour.

4.2.4.8.4 At the end of 24 hours, the resulting solution should be titrated to a pH between 10 and 12. Test for presence of active chlorine by use of acidic potassium iodide solution give free iodine color. If negative, add additional 5.25% NaOCl solution, wait for two hours, then test again for active chlorine. Continue procedure until positive chlorine is given by solution.

4.2.4.8.6. Alternate solutions for the decontamination of HD.

4.2.4.8.6.1. HD is decontaminated with the 10% $Ca(OCl)_2$ solution (4.2.4.1.4). HD is poorly soluble in water.

4.2.4.8.6.2 A minimum of 65 grams of decon solution is required for each gram of HD.

4.2.4.8.6.3 Decontaminant/agent solution is allowed to agitate for a minimum of one hour. Agitation is not necessary following the first hour.

4.2.4.8.6.4 At the end of 24 hours, the resulting solution should be titrated to a pH between 10 and 12. Test for presence of active chlorine by use of acidic potassium iodide solution give free iodine color. If negative, add additional 10% $Ca(OCl)_2$ solution, wait for two hours, then test again for active chlorine. Continue procedure until positive chlorine is given by solution.

4.2.5. Analysis.

NMR analysis is performed to characterize the products formed, however the techniques are not sensitive enough to determine trace amounts of agents (< 0.5%).

4.2.5.1 ^{31}P NMR.

The GA, GD, and two VX decon products will be analyzed by ^{31}P NMR. Samples will consist of 0.5 to 1 mL in 5 mm NMR tubes. Spectra will be recorded on a Varian XL-200 Superconducting FT-NMR System operating at 81 MHz in an unlocked mode. Spectra will be obtained at probe temperature (ca. 21°C), using phosphoric acid (85%) as the external reference. The chemical shift values (δ) determined are good to better then - 0.1 ppm. Data will be accumulated from 2 to 18 hours depending on signal-to-noise levels. All

spectra will be obtained using a pulse width of 3 µsec (33 degree), a sweep width of 20 KHz, an acquisition time of 1.6 sec, and a pulse delay of 2.5-3.3 sec. Grated decoupling will be used to eliminate any nuclear Overhauser effects, and quantitative data will be obtained by digital integration of the peak areas. Detectable limit: 200µg of agent/mL of original decon solution.

4.2.5.2 ^{13}C NMR.

The HD decon product will be analyzed by ^{13}C NMR. Samples will consist of 0.5 to 1.0 mL in 5 mm NMR tubes. Spectra will be recorded on a Varian XL-200 Superconducting FTNMR System operating at 50 MHz in an unlocked mode. Spectra will be obtained at probe temperature (ca. 21°C), using tetramethylsilane (TMS) in chloroform as the external reference. The chemical shift values (δ) determined are good to better then -0.1 ppm. Data will be accumulated from 2 to 18 hours depending on signal-to-noise levels. All spectra will be obtained using a pulse width of 3.5 µsec (33°), a sweep width of 12 KHz, an acquistion time of 1.6 sec, and a pulse delay of 2.5-3.0 sec. WALTZ decoupling will be used for full proton decoupling, and quantitative data will be obtained by digital integration of the peak areas. Detectable limit: 0.5-1mg of agent/mL of original decon solution.

4.2.5.3 Potassium iodide test for active chlorine.

Place roughly 3 mL decon solution in a small erlenmeyer flask. Add several crystals of potassium iodide and swirl to dissolve. Using a small graduated cylinder rapidly add about 3 mL of a 50 wt.% sulfuric acid/water and swirl. An immediate iodine red color shows the presence of active chlorine. (NOTE: A gradual appearance of red indicates air oxidation of the potassium iodide and not chlorine. To be considered positive the red color must appear immediately upon addition of the acid mixture.)

4.2.6. Data Storage

Test data will be recorded in official CRDEC notebooks.

The data recorded will include:

a. Complete record of chemical substances used to include lot number and manufacturer.

b. Starting purity of the chemical agents.

c. Quantities of chemicals and agents used.

d. Time allowed between adding agent and neutralizing decon.

e. pH after decontamination step and after neutralization step.

f. Analysis of products.

g. Record laboratory temperatures during decon step.

i. Any problems that arise and how problem was solved.

j. Any changes necessary to the procedures spelled out in this protocol will be documented.

4.3 Toxicology Protocols - Current

4.3.1 INTRODUCTION

The Good Laboratory Practices (GLPs) were the first significant regulations implemented without a preceding catastrophe that had impacted on the health and safety of the American public. Although considerable data were falsified and misrepresented prior to the implementation of the GLPs, there is no documentation that products registered or approved using the faulty data, caused any significant harm to the public.

The first Pure Food and Drug Law passed in 1906 resulted from the contamination and filth exposed in the meat packing industry (see Upton Sinclair, The Jungle), foods adulterated with chemical preservatives (Dr. Harvey Wiley's Poison Squad), and quack remedies. This law, also known as the Wiley Act, prohibited the manufacture and interstate shipment of adulterated and misbranded foods and drugs.

In 1938 the Federal Food, Drug, and Cosmetic Act (FFDCA) was passed, prompted in part from the Elixir Sulfanilamide episode in 1937 that had resulted in over 100 deaths. Although sulfanilamide tablets and powder had previously been used safely and effectively for the treatment of streptococcal infections, the elixir, in diethylene glycol (antifreeze) had not been tested for toxicity, nor was it a requirement by law at that time. The 1938 FFDCA required that drug manufacturers provide scientific proof that new products were safe for use before they could be marketed.

Following the thalidomide tragedy in Europe, where thousands of deformed infants were born to mothers who had taken this new drug, the 1962 Kefauver-Harris amendment was passed. This served to strengthen the FFDCA by requiring that not only safety had to be demonstrated for any new drug, but also efficacy. Additionally, adverse reactions were to be reported, advertising had to be accurate and complete, and Good Manufacturing Practices Regulations (GMPs) were established. The GMPs set standards for plant facilities, their maintenance, and laboratory controls in an attempt to prevent errors or accidents that could harm consumers.

Compliance with GLPs, although cumbersome to implement and considered a nuisance and waste of time by some study directors or principal

investigators, can really be advantageous in the management and validation leading to the acceptance of the study by regulatory agencies and peers.

The GLPs are here to stay and most laboratories have or are implementing them. The key to their successful implementation and execution is to participate, document, and validate. The most recent GLPs of December 28, 1987 are in Appendix 8.2.1.

4.3.2 TOXICOLOGY DIVISION GLP COMPLIANCE MEASURES

The management of Toxicology Division established a Quality Assurance Unit to implement Good Laboratory Practice standards in all studies performed by the division. QAU personnel consists of two individuals who are responsible for many tasks relating to each study. The GLP standards impact many areas including : management's responsibility, maintenance of the physical plant, (especially animal care and laboratory facilities), personnel, equipment maintenance and calibration, SOP's , test and control substances characterization, handling and storage, the study protocol and conduct, and reporting and record retention.

The specific tasks assigned to the Quality Assurance Unit are detailed below. The protocol for the study is reviewed by the QAU to assure that the study director has suitably addressed the study parameters listed in the act. All protocols are maintained in the office of the QAU while studies are in progress. Copies of Standard Operating Procedures (SOP) for laboratory and facility operations are reviewed when prepared and maintained by the QAU. A Master Schedule is maintained which lists and details pertinent information for each study. The schedule is periodically updated and treated as raw data. All studies are subject to inspection of critical phases by the QAU. The inspections are beneficial in identifying problems and recommending actions to resolve them. They also serve as a mechanism to notify management when problems exist which may affect the integrity of the study. The QAU is tasked with determining that no deviations from approved protocols and/or SOP's were made without proper documentation. The final report of the study is reviewed by the unit to assure that the report describes the methods used to generate data and that the results reported accurately reflect the raw data. The standards require the QAU to prepare a statement for inclusion in the final report which documents when inspections were made and the findings of the same reported to management and the study director. An archive for retention of raw data and documents required to validate the study is maintained by the QAU. The statute mandates retention of records for at least ten years following conclusion of the study.

4.3.3 ARCHIVE RECORDS

CRDEC has reviewed the toxicology data base of unpublished data going back to the early 1960's to determine its applicability to address the current issues.

The data collected and presented in summary form are found in Appendix 8.2.2. This table indicates that the lethal dose 50 (LD_{50}) of the test samples are greater than those considered hazardous waste. This indicates that decontaminated solutions tested were less toxic than the COMAR criteria 10.51.02.08. The methods used to generate the toxicity data in our laboratories follow the guidelines as described by the Department of Transportation (DOT), the Food and Drug Administration (FDA) Federal Hazardous Substance Act (FHSH). For the current tests, the protocols utilized at CRDEC are contained in the Appendix and conform to the Department of Transportation requirement for class "B" poison, also defined in Appendix 8.2.4. The detailed chemical reactions of the current standard operating procedure for decontamination/detoxification are described in the Sections 4.1 and 4.2 above.

Decontamination procedures for lethal chemical warfare agents have been fully developed by the research staff of CRDEC. A comprehensive literature survey is presented in Sec. 4.1. This survey covers the experimental decontamination procedures examined in the laboratories for the destruction of chemical agents. Edgewood Arsenal Technical Report EATR-4755 (Owens, et al, 1973) utilized some of the experimental methods described in the literature survey on deconned agent samples provided by the Chemical Process Laboratory and the resulting toxicities were determined.

Lewisite, an arsenical vesicant produced for World War II, subjected to basic solutions decomposes to the inorganic arsenite, chloride and acetylene. The chemical literature of the early 1940's reported these studies in detail, Waters and Willians (1950). The chemical literature of the organometallic arsenic compounds of Lewisite has been reviewed by Doak and Freedman (Organometallic Compounds of Arsenic, Antimony and Bismuth, John Wiley & Sons, Inc., New York, 1970, p. 65, 89- 90, 103-104, 109-110).

In practice, to assure fast and complete destruction of the toxic agents, a large excess of the decontaminant is used, so that, the agent is always present in concentrations that would assure the reactions would proceed with rates similar to, if not the same as, the first order reaction rates. Before the wastes are disposed of they are checked to insure that active decontaminant remains in the spent solutions. These waste are then transferred by a number of mechanisms (i.e. transfer in steel drums or by closed waste drain lines) to a waste collections system for ultimate disposal. We have found no record of adamsite (K996) use or decontamination, however, incineration appears to be the detoxification method of choice. There was also no toxicological data found for decontaminated GD (K993). However, its chemistry is well defined and detoxification is accomplished by hydrolysis.

Decontaminated GA (K991) (Appendix 8.2.2) indicates that the LD_{50} is less than 50 mg/kg by the oral route suggesting it is a hazardous substance orally, but not dermally. Our records show that GA in this experiment was detoxified to produce sodium cyanide which could account for the oral toxicity. The standard procedure now used at CRDEC provides for destruction of the cyanide by oxidation with sodium hypochlorite subsequent to hydrolysis.

Agent T (K998) is a mustard and was not used as a separate filling in munition. However, agent T is found as a mixture/solution with HD and is listed as HT. Therefore, separate agent T (K998) is not applicable and should be delisted.

4.3.4 RESULTS OF CURRENT TESTS

Recently CRDEC identified three decon solutions for further testing. These were VX/10% $Ca(OCl)_2$, GD/10% NaOH, and HD/5.25% NaOCl and were tested in rabbits dermally and in rats by the oral and inhalation routes according to the protocols in the Appendix. These 48 hour DOT tests require that if 4 or fewer of a group of 10 animals (5 of each sex) die during the test, the test is negative. That is, it is less than a class B poison. If 5 or more die, the material would be listed as a class B poison, and thus a hazardous waste.

The dose levels used were 0.2 mL/kg dermally in rabbits, 0.05 mL/kg orally in rats, and a target concentration of 2 mg/kg by inhalation in rats.

Results: None of the animals died in these tests. The results are presented in the following table.

Materials and 48 Hour Mortality

Species (route)	Dose mL/kg	VX/$Ca(OCl)_2$ # Dead/#Tested	GD/NaOH # Dead/#Tested	HD/NaOCl # Dead/#Tested
rabbit[1] (dermal)	0.2	0/10[a]	0/10	0/10[b]
rat[1] (oral)	0.05	0/10	0/10	0/10
rat[2] (inhal)	10mg/L (M)	0/5[c]		
	10mg/L (F)	0/5[c]		
	6mg/L (M)		0/5	
	7mg/L (F)		0/5	
	13mg/L			0/5[c]
	18mg/L			0/5[c]

[a] Mild skin irritation observed in 4 of 10 rabbits prior to 24 hours with VX/$Ca(OCl)_2$ which disappeared by 48 hours.

[b] Mild skin irritation observed in 10 of 10 rabbits after 24 hours with HD/NaOCl and persisted for 48 hours.

[c] During exposure to VX/$Ca(OCl)_2$ and HD/5.25% NaOCl the rats appeared to be lethargic and showed signs of mucous membrane irritation. These effects were present only during the exposure.

[1] Notebook reference 88-0005, pages 4-13.

[2] Notebook reference 87-0128, pages 18-33.

Note: Although no deaths occurred in any of the tests conducted, the concentrations generated for the inhalation exposures far exceeded the target of 2 mg/L and may in part explain the observations reported.

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5.0 ANALYTICAL METHODS

5.1 Introduction

Decontamination studies generally are conducted to determine the rate of reaction of a chemical warfare agent with a known decontaminant in a well defined media. These studies require that the systems under investigation be amenable to analysis of either the starting materials or their reaction byproducts. In most cases the rate of disappearance of the chemical agent was selected since oxidation reactions in general are difficult to study by examining the reaction byproducts. The analytical methods employed were calibrated for the systems and would not as a general rule be methods of analysis for a broad spectrum of conditions. Methods such as Ultraviolet adsorption spectrometry were commonly used, since the chemical agents have an absorption and the reaction products do not. These techniques were especially useful is studying the very fast reactions indicative of the G-Agents with strong basic solutions. When the Chemical Demilitarization Project Officer (Demil PO) was established in the late 1960's, these methods of analysis used for decontamination studies were not acceptable to the regulatory agencies established to oversee and approve these disposal operations. As a result CRDEC in concert with the Demil PO, investigated a number of alternate methods of analysis suitable for these operations. Due to the uniformity of demil operations, the analytical methods used, to control and validate these disposal process, could be tailored and made specific for the disposal process. The methods reviewed in this presentation cover classical visible/ultraviolet, fluorescence and enzymatic colorimetry, chromatography including thin layer, high performance liquid, and gas/liquid, in addition to specialized methods used in the demilitarization operations. No review of analytical methodology would be complete without adequate discussion of the sampling and clean-up procedures, and some assessment of the quality of the results based on established standards and good laboratory practices.

5.2 Chromatographic Analysis

5.2.1 Gas/Liquid Chromatography (GLC):

A review of analytical procedures for GB and VX by Crabtree and Sarver[5-1] provides an acceptable discussion of using this instrumental methods of analysis. There has been little change in the instrumentation since this review. Several unique arrangements of specific detectors have improved there sensitivity; however, these improvements are not measured in orders of magnitude, but within the same order. Packed column technology was used routinely at time of the review and not references to capillary wall coated open tube (WCOT) colums were available at that time. This advancement has been incorporated into most of the methods used in the demil operations. Southern Research under contract to U.S. Army Toxic and Hazardous Materials Agency (THAMA) developed a series of methods for analyzing the chemical warfare agents GB, VX, and HD[5-2].

5.2.2 Sampling and Clean-up Techniques for GLC:

Detection and quantification of the Chemical Warfare Agents GB, VX, HD, and L in strong solutions, used for laboratory decontamination, where the agent is always presence in vanishingly small quantities presents unique sampling problems. In order to demonstrate the effectiveness of the sampling procedure and ultimately the decontamination process, a modified quench process is used. This process requires neutralization of the decontaminant followed by extraction of the neutralized solution with an agent laden extraction solvent. If the agent is then recovered and quantified, the analytical method is considered to be adequate to determine residual agent in the neutralized decontaminant. In the case of GB, salt has to be added to the neutralized decontaminant to saturation so that the extraction efficiency (64%)[5-2c] is favorable to the organic solvent. Procedures for doing this type analysis for GB, VX, and HD are given by Smith and Fowler[5-2]. In the late 1970's, Southern Research under contract to THAMA developed a sries of air monitoring systems based on the use of solid sorbent bed sampler, the Automatic Chemical Agent Air Monitoring System (ACAMS).[5-3b] This effort along with those under the CRDEC Depot Area Air Monitoring System (DAAMS) program have lead to the development of highly sensitive and specific methods of analysis for the CW agents, HD, GB, and VX. Employment of these methods require the modification of the Injection Port of commercially available GLC's. Once this has been accomplished the extract is loaded on the sorbent bed by aspiration from a glass wool plug containing the agent laden extract. The plug is removed, and the glass tube containing the sorbent bed is thermal blackflushed by the GC carrier gas onto the analytical GLC column. For the listed CW agent HD, GB, and VX, the gas chromatograph is equipped with a Flame Photometric Detector (FPD). These methods work well in the low to medium ultra-trace (PPB) levels. At present no equivalent method of analysis is available for Lewisite, however, several excellent projects carried out by S. F. Hallowell and P. C. Bossle have resulted in numerous approaches using both GLC and other types of chromatography and instrumentation that could ultimately lead to a specific and sensitive method of analysis[5-6].

5.2.3 Other Chromatography Methods:

5.2.3.1 Thin Layer Chromatography. Crabtree and Sarver[5-1] contains a good review of this method. Due to the inherent lack of good sensitivity of this method, no further discussion is appropriate.

5.2.3.2 High Performance Liquid Chromatography Methods. Bossle[5-7, 5-8 and 5-9] has developed a direct method of analysis for Mustard, HD, and Lewisite, L, and hydrolysates in aqueous solutions which is effective in low PPM's to the high PPB's. These methods are based on reverse phase chromatography using spectrophotometric and electrochemical detection.

5.3 References

5-1. Crabtree, E. V., Sarver, E. W. EC-TR-76021. Review of Analytical Procedures for GB, VX, and Their Degradation Products. Jan 1977 (U).

5-2. Smith, J. E., Jr., and Fowler, W. K. Analytical Methods Development - Volume 2 - Standing Operating Procedures. Contract DAAK11-82-C-0162 Final Report. Dec 1985 (U); b. Benson, J. H., etal. EM-TR-73070. Laboratory and Pilot Scale Detoxification of VX in Acidic Media Using Chlorine Gas, May 1974.; c. Mohrman, G. B., Rocky Mountain Arsenal SOP NO: SARRM-TOE-17. Index for Low Level Sarin (GB). MAR, 1979.

5-3. a. Duggan, M. L. ARCSL-TR-78048. Pilot-Scale Incineration and Detection Interference Studies for the Disposal of Toxic Gas and War Gas Identification Set; b. Sides, Gary D., Contract DAAK11-77-C-0075. Construction, Development, and Testing of an Automatic Continuous Air Monitoring System (ACAMS) for Use at the Chemical Agent Munitions Disposal System (CAMDS), Dec 1984.

5-4. Soderquist, C. J., Crosby, D. G., and Bower, J. B. Determination of Cacodylic Acid (Hydroxydimethylarsine Oxide) by Gas Chromatography. Anal. Chem. 46, 155-157, (1974).

5-5. Sarver, E. W. Unpublished Results (1974).

5-6. Hallowell, S. F., and Bossle, P. C. Personal Communication and Unpublished Results (1980-1986).

5-7. Bossle, P. C., Martin, J. J., and Sarver, E. W. High Performance Liquid Chromatography Analysis of 2-Chloroethyl Ethylsulfide and its Decomposition By-Products by Derivatization. J. Chromatog. 263, 412-416 (1984).

5-8. Bossle, P. C., Hallowell, S. F., Reutter, D. J., and Sarver, E. W. The analysis of 2,2'-Thioethanol, a Water Soluble Alkyl Sulfide in Aqueous Matrices by LCEC. J. Chromatog. 330, 388-391 (1985).

5-9 Bossle, P. C. Determination of Lewisite Contamination in Environmental Waters by HPLC. Rocky Mountain Analytical Conference, 31 JUL - 5 AUG 1988, Denver, CO.

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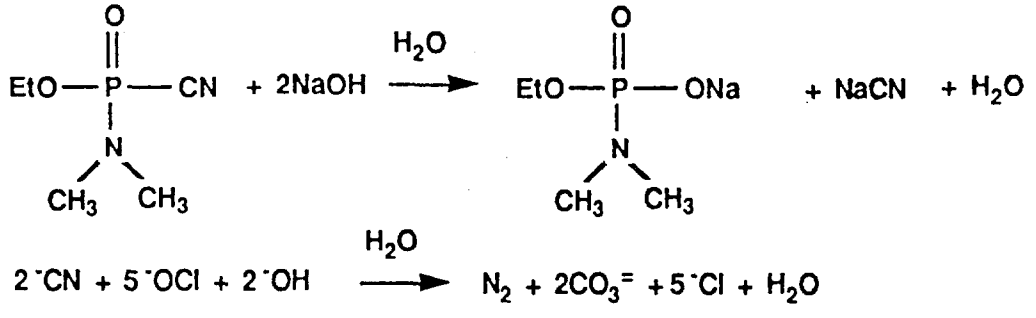
6 Summary of Data

Introduction

In practice, to assure fast and complete destruction of the toxic agent(s), a large excess of the decontaminant is always used; thus the agent is always present in concentrations that would assure the reactions would proceed with rates similar to, if not the same as, the first order reaction rates.

6.1 Ethyl dimethylamidocyanophosphate (GA, Tabun)

GA is routinely decontaminated with 10% sodium hydroxide solution (see Section 4.2). This reaction is extremely fast (estimated half-life < 5 sec) and rapidly releases cyanide. The second step in the decon procedure is the well known reaction of cyanide with hypochlorite (Kirk-Othmer Encyclopedia of Chemical Technology. 3d Edition. Vol. 7 John Wiley and Sons, New York, New York, 1979, pages 316-7) to form nitrogen gas. Thus the overall decon reaction takes place in two steps according to the following reaction:

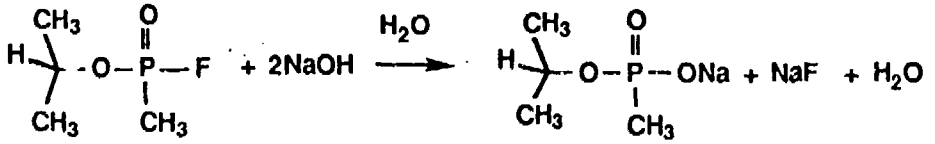


GA is also rapidly deconned (10 half-lives of destruction in 30 minutes or less) using sodium carbonate solutions, aqueous solutions of hypochlorite, and alcoholic sodium hydroxide (see Appendix 8.1.9-8.1.12. Please note that the nmr experiment as presented is designed to show the products of phosphorous hydrolysis. Although routine procedure is to treat the solution with hypochlorite following hydrolysis in the second step, this nmr experiment is not designed to observe cyanide. Thus the equation showing the stoichiometry of the reaction in 8.1.9-8.1.12 is representative only of the first step, the procedure where the measurement is valid.). The decontamination is also quite rapid when calcium

hypochlorite is used in conjunction with organic emulsions (the German C8 emulsion). Toxicological tests (see Appendix) of GA detoxified waste show Oral LD_{50} values < 50 mg/kg (Rat, probably due to presence of cyanide in the solution tested) and Dermal LD_{50} values of > 200 mg/kg (Rabbit). The present procedure at CRDEC is to treat the decon solution, following hydrolysis, with hypochlorite to destroy the cyanide.

6.2 Isopropyl methylfluorophosphonate (GB, Sarin)

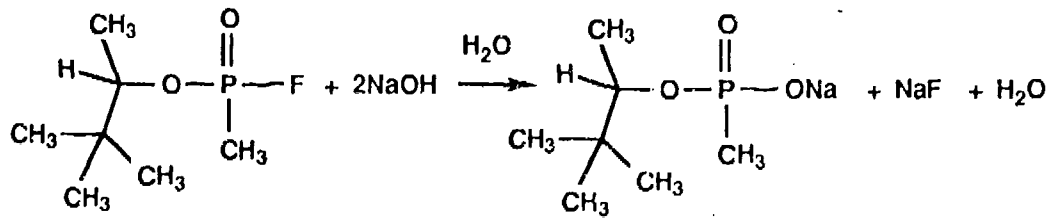
Extensive data exists on the decontamination of GB. This reaction is extremely fast (estimated half-life < 5 sec) and rapidly releases fluoride according to the following reaction:



GB is routinely decontaminated using 10% sodium hydroxide, 10% sodium carbonate, 5.25% sodium hypochlorite, neat monoethanolamine, and by a 25% aqueous monoethanolamine. The decontamination is also quite rapid when calcium hypochlorite is used in conjunction with organic emulsions (the German C8 emulsion). Toxicological tests (see Appendix) of GB detoxified waste show Oral LD_{50} values > 50 mg/kg (Rat) and Dermal LD_{50} values of > 200 mg/kg (Rabbit). Because of the extensive work in the Demil program with GB, additional tests show Oral LD_{50} values 566 mg/kg (Rat- 24 hr), 271 mg/kg (Rat- 14 day) and Dermal LD_{50} values of > 200 mg/kg (Rabbit). In all cases nmr analysis of product formation (see Appendix) confirmed earlier work showing the speed of decontamination (10 half-lives < 5 minutes).

6.3 Pinacolyl methylfluorophosphonate (GD, Soman)

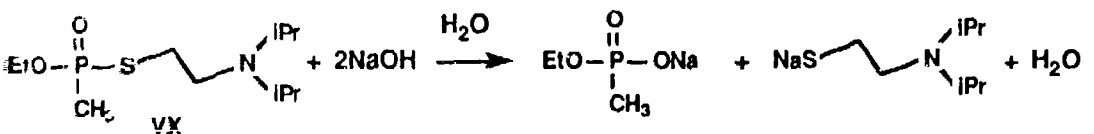
The chemistry of GD is very similar to that of GB, both in reaction kinetics and product formation. The product phosphonic acid differs from the GB products only in the alcohol portion (pinacoyl rather than isopropyl) according to the following reaction:



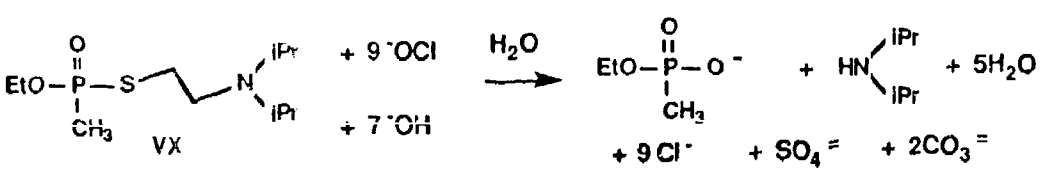
GD is routinely decontaminated using 10% sodium hydroxide, 10% sodium carbonate, 5.25% sodium hypochlorite, neat monoethanolamine, and by a 25% aqueous monoethanolamine. The decontamination is also quite rapid when calcium hypochlorite is used in conjunction with organic emulsions (the German C8 emulsion). There was no extensive toxicological data found for decontaminated GD (K993). However, its chemistry is well defined when detoxification is accomplished by hydrolysis. Toxicological tests (see Appendix) of aqueous hydroxide GD detoxified waste show Oral LD_{50} values > 50 mg/kg (Rat) and Dermal LD_{50} values of > 200 mg/kg (Rabbit). In all cases nmr analysis of product formation (see Appendix) confirmed earlier work showing the speed of decontamination (10 half-lives < 5 minutes).

6.4 O-Ethyl-S-(2-diisopropylaminoethyl) methylphosphonothioate (VX)

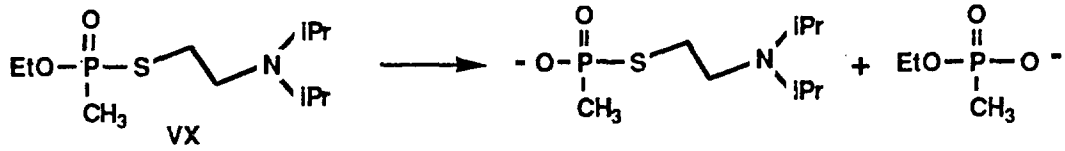
VX is more resistant to cleavage by bases than GA, GB, and GD and treatment with strong base (hydroxide) and/or hypochlorite for longer reaction periods is the recommended procedure. This reaction is moderate (estimated half-life < 900 sec) and releases the thio fragment in a similar mechanism to that observed for GA, GB, and GD, as shown below:



One major concern with VX is its reduced solubility in aqueous solutions, thus alcoholic co-solvents are recommended during the decontamination reaction of quantities greater than 50g. Treatment of dilute VX with aqueous hypochlorite reacts at phosphorous to give ethyl methylphosphonic acid, shown in the reaction below:



In the reaction of VX with alcoholic 10% sodium hydroxide the hydrolysis is similar to hydrolysis using hypochlorite. In extensive studies related to large scale Demil procedures, treatment of VX produces the biproduct from the "bis' impurity to yield waste which is initially tested as toxic by intravenous injection, weakly toxic by oral administration, but exhibits no dermal toxicity. The biproduct is a non-volatile crystalline solid, miscible in water and alcohol, and was not considered a vapor hazard. Thus no inhalation experiments were performed. The reaction is thought to occur as shown:



VX

Data at CRDEC indicates that this "bis" impurity is formed to the extent of about 10% during alcoholic base hydrolysis and is slowly hydrolyzed at pH > 13, thus even when formed it is converted to the less toxic fragments from cleavage of the P-S bond. Use of the acid chlorinolysis procedure (suitable for large scale decontamination related to Demil, but difficult to perform safely on a laboratory scale) yields a clean hydrolysis product with cleavage at the P-S bond of VX to produce decontamination products which contain no "bis" impurity and which test non-toxic in animal screens. Exposure of VX to excess base and hypochlorite (as outlined in Section 4.2.4.6) indicates that the "bis" impurity is not formed during the reaction. A solution used for toxicological tests resulting from decontamination of VX with 10% calcium hypochlorite according to the protocol given in Section 4.2.4.6 showed > 99.5% VX destroyed (nmr analysis) and the products are the expected ethyl methylphosphonic acid salt (again nmr analysis) and no "bis" impurity or "pyro" signal. This solution, when tested in the standard toxicological protocol (Section 4.3.4) is non-toxic (See also Appendix). Therefore the recommended decon for VX, as outlined in Section 4.2.4.6, is for reaction of VX with calcium hypochlorite solutions.

6.5 Chlorovinylarsine dichloride (Lewisite)

Lewisite, an arsenical vesicant produced for World War II, subjected to basic solutions decomposes to the inorganic arsenite, chloride and acetylene. Lewisite, in 10% aqueous base, is extremely fast (estimated half-life < 5 sec) and rapidly releases acetylene and inorganic arsenite according to the following reaction:

$$ClCH{=}CHAsCl_2 + 6\ NaOH \rightarrow Na_3AsO_3 + 3\ NaCl + H_2C_2 + 3\ H_20$$

The chemical literature of the early 1940's reported these studies in detail, the classic work being that of Waters and Willians (1950). The chemical literature of the organometallic arsenic compounds of Lewisite has been reviewed extensively by Doak and Freedman (Organometallic Compounds of Arsenic, Antimony and Bismuth, John Wiley & Sons, Inc., New York, 1970, p. 65, 89- 90, 103-104, 109-110). This base reaction is so rapid that it is difficult to analyze a decon solution of Lewisite rapidly enough by most analytical techniques to show any residual agent. Preparation of the decon reaction in preparation for nmr analysis immediately causes effervescence (acetylene production) followed by a spectrum that contains no Lewisite and also no organic signals (See Appendix). In all cases nmr analysis of product formation (see Appendix) confirmed earlier work showing the speed of decontamination (10 half-lives < 5 minutes). Since there has been very little interest in this agent since WWII little

recent work at CRDEC has been performed and a minimum of waste is generated.

6.6 Phenarsazine chloride (Adamsite)

We have found no record of adamsite (K996) use or decontamination at CRDEC, however, incineration appears to be the detoxification method of choice.

6.7 Bis(2-chloroethyl) sulfide (HD, Sulfur Mustard)

As stated in the review of mustard chemistry above (Section 4.1), this agent reacts with water and aqueous hydroxide at similar rates and this procedure is not considered acceptable. The only exception to this general rule is the standard use of DS2 for field expedient decontamination. This base reaction reacts rapidly with mustard to form divinyl sulfide with a half-life of < 30 sec. This solution is not usually used form deliberate decon as its capacity is low and it is extremely corrosive to equipment, although the method is fast. Since WWI the preferred way to decontaminate solutions of mustard or mustard analogues is to oxidize them with chlorine oxidants. The use hypochlorite oxidation is rapid and characterized by production of numerous products. Nitric acid oxidation, suggested in the archival literature, rapidly produces only mustard sulfoxide as its decon product. Because of expense, kinetics and ease of use hypochlorite oxidation is now the standard deliberate decon method used against mustard. Nmr analysis of mustard decontaminated with hypochlorite show at least 20 products, however, greater than 99.5% destruction of mustard. Numerous studies have shown that in the presence of excess chlorine oxidant that mustard is rapidly destroyed to multiple products which are non-toxic in the usual toxicological tests (see Appendix).

6.8 2,2'-di(3-chloroethylthio)-diethylether (T)

Agent T (K998) is a mustard and was not used as a separate filling in munitions. However, agent T is found as a mixture/solution with HD and is listed as HT. Although T has not been studied as a separate agent, its chemistry is very close to mustard and therefore all the discussion applicable for H should be comparable to T (A similar situation exists in comparison of GB with GD. Although a slightly different structure is involved the reactions are found to be very similar and usually differ only in solubility parameters and slight differences in kinetic rates.).

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7.0 Summary

In September 1987, the State of Maryland requested that CRDEC demonstrate that residues from decontamination of chemical warfare agents used in research contain no chemical agents. This information would be used to make a determination as to whether the decontamination procedures resulted in wastes than can be excluded from current regulation.

Specifically, the State of Maryland requested a detailed description of the actual decontamination procedures used on laboratory materials, including step-by-step outline of the decontamination process, identity of the specific decontaminating solution used for a given CW agent, the theoretical chemical basis for a given decontaminating action, and the concentrations, time and any other parameters that influence the degree which the reaction goes to completion. In addition, the State requested documentation that decontaminated wastes used for toxicology tests are equivalent to those resulting from the actual decontamination process and well as documentation that toxicological tests follow generally accepted practices.

In response to these issues this document supports the basic premise that these decontamination reactions are well understood and documented. Do theoretical chemical calculations support claims that agents plus decontaminants yield products that no longer contain agents? They do. Reaction energies, reaction kinetics, chemical equilibrium, laws of thermodynamics, material balance and other mathematical considerations indicate that A + B do indeed equal C + D (Section 2). In addition, product analysis procedures indicate that the quantity of CW agents remaining following decontamination are below detectable limits (Section 5) and new analytical techniques are constantly being incorporated to improve the sensitivity, accuracy and speed of these determinations.

Are older decontamination procedures, which used different reagents, equivalent to today's protocols and reagents? In most cases, yes. For example, when using sodium hydroxide or sodium carbonate, the reactive decontaminating moiety in both cases (see discussion in Section 3) is the hydroxyl ion (^-OH). In the search for the most efficient procedures, numerous systems have been investigated (extensively documented in Section 4.1). Over the years several procedures have been shown to be consistently efficient against a broad range of agents, and these procedures are now the accepted "standard" for routine decontamination, although the search continues for more efficient techniques. Incorporated into this document are specific examples of the procedures utilized in present decontamination procedures against listed CW agents (Section 4.2).

Do analytical results and toxicological data substantiate complete destruction of chemical agents when decontaminated? Yes. Extensive information accrued since 1918 provides incontrovertible scientific evidence of decontamination efficacy. As can be seen from the specific examples given in Section 4, information exists on procedures where the decontamination solution

are analyzed both chemically and *via* toxicological methods. Those solutions, which are certified to contain no remaining CW agents are the ones which also show minimal toxicity in toxicological examination accepted by the industrial and regulatory communities. Thus, both chemically and toxicologically, these processes have demonstrated that no CW agent remains.

The extensive decontamination experience and comprehensive data bases at CRDEC have underwritten huge demilitarization projects in the past. In all cases, decontamination and disposal projects for agent-filled munitions were executed safety, without untoward incident, and in total compliance with every prevailing environmental and human safety stricture and concern. In addition, the concern for ensuring safety has lead to adoption of large margins of safety into these procedures. Once theoretical parameters are determined, excess decontaminant is utilized as a margin of safety. Therefore, protocols today are even safer than those used in previous years. These and other facts enumerated in detail in this document provide ample evidence that current decontamination protocols and procedures are safe, scientific, and result in the total destruction of chemical agents.

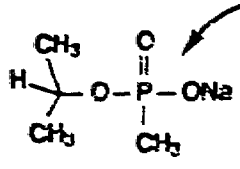
APPENDIX

8.1 NMR Data

8.1.1 GB Hydrolysis in 10% NaOH

Experimental Conditions: 0.02 mL of GB in 1 mL of 10% NaOH (2.5N): pH > 14
Sample miscible with shaking.

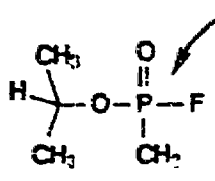
Results: Spectrum at ~ 10 min shows only:



^{31}P at 26.3 ppm

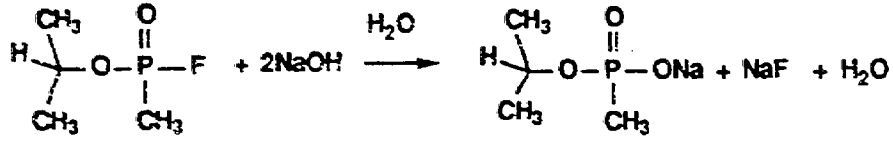
Upper Trace: Hydrolysis Product

Lower Trace: GB Starting Material

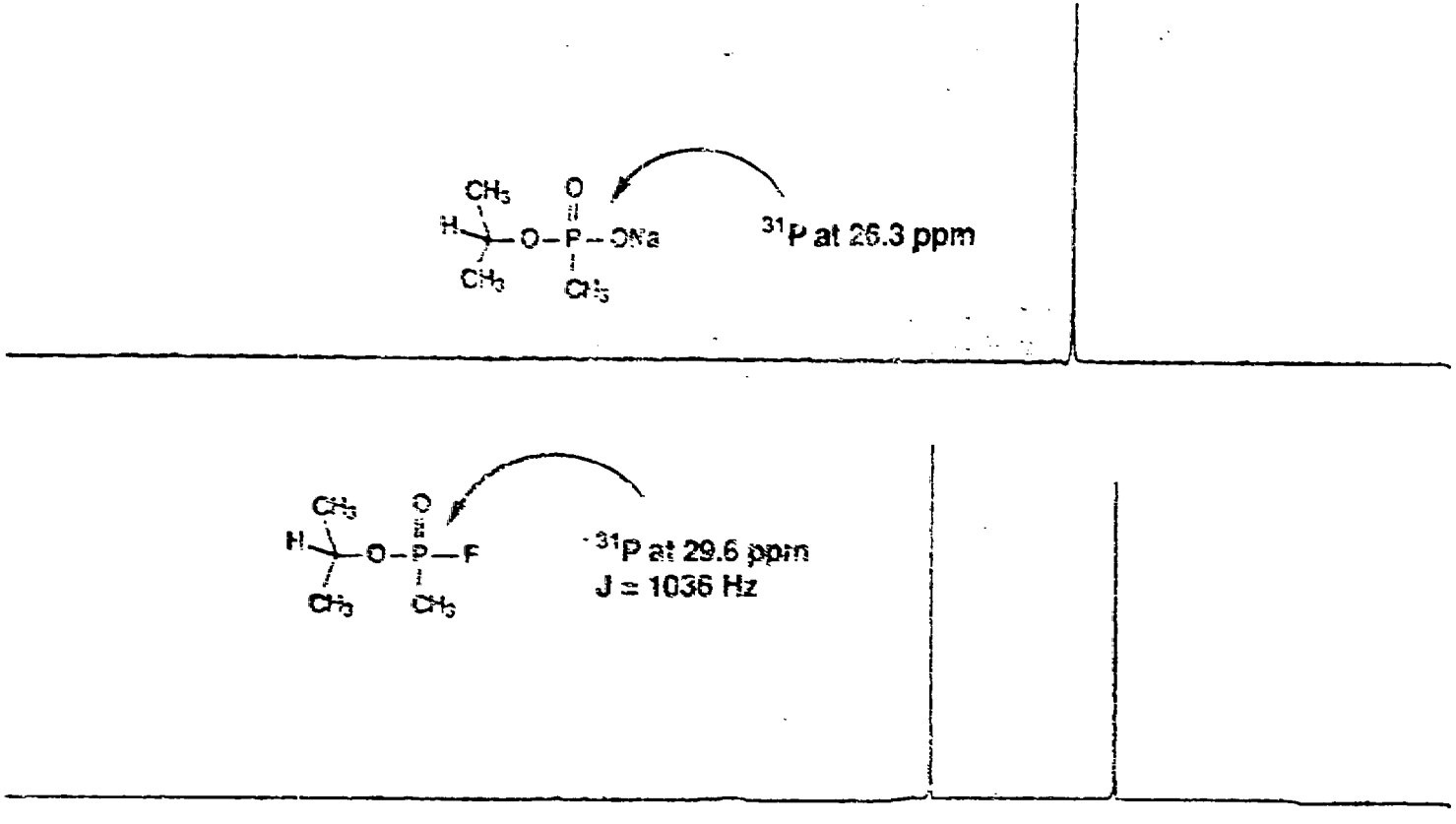


^{31}P at 29.6 ppm
J = 1036 Hz

Hydrolysis Stoichiometry:



8.1.2 GB Hydrolysis in 10% Na_2CO_3

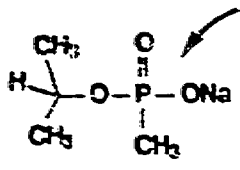


$$\text{GB} + 2Na_2CO_3 \xrightarrow{H_2O} \text{GB-Na} + NaF + H_2O + 2NaHCO_3$$

8.1.3 GB Hydrolysis in 10% Alcoholic NaOH

Experimental Conditions: 0.02 mL of GB in 1 mL of 10% Alcoholic NaOH (2.5N): pH > 14
Sample miscible with shaking.

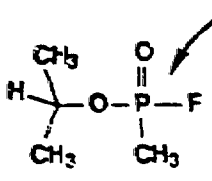
Results: Spectrum at ~ 10 min shows only:



^{31}P at 25.9 ppm

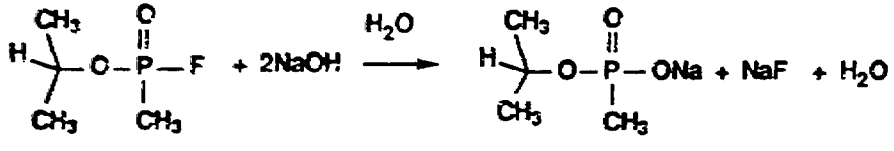
Upper Trace: Hydrolysis Product

Lower Trace: GB Starting Material

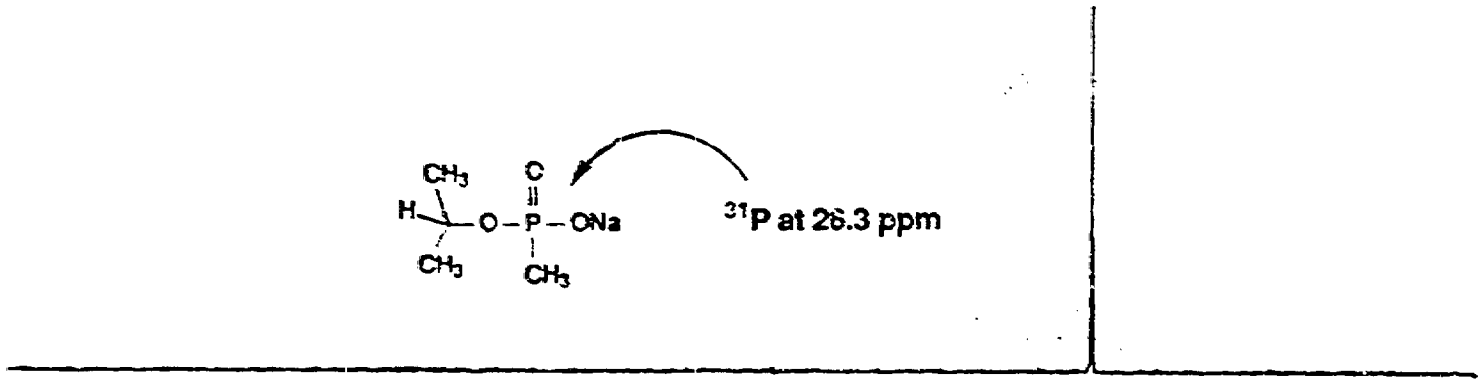


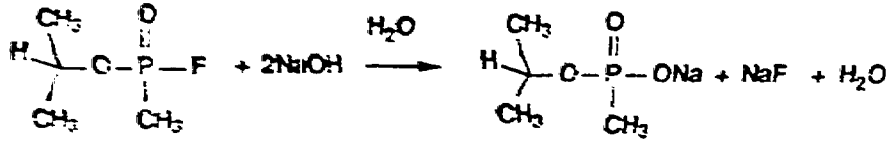
^{31}P at 29.6 ppm
J = 1036 Hz

Hydrolysis Stoichiometry:



8.1.1 GB Hydrolysis in 10% NaOH

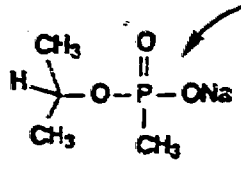




8.1.2 GB Hydrolysis in 10% Na_2CO_3

Experimental Conditions: 0.02 mL of GB in 1 mL of 10% Na_2CO_3 (2.0N): pH 12.2
Sample miscible with shaking.

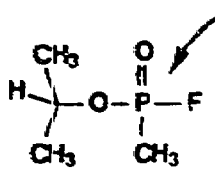
Results: Spectrum at ~ 5 min shows only:



^{31}P at 26.3 ppm

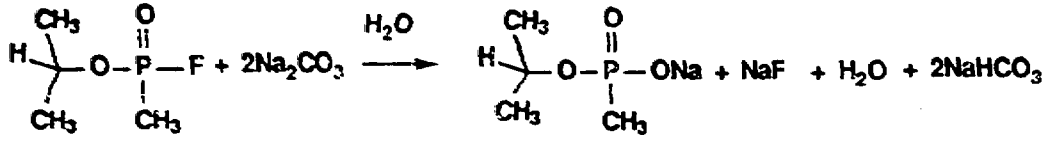
Upper Trace: Hydrolysis Product

Lower Trace: GB Starting Material

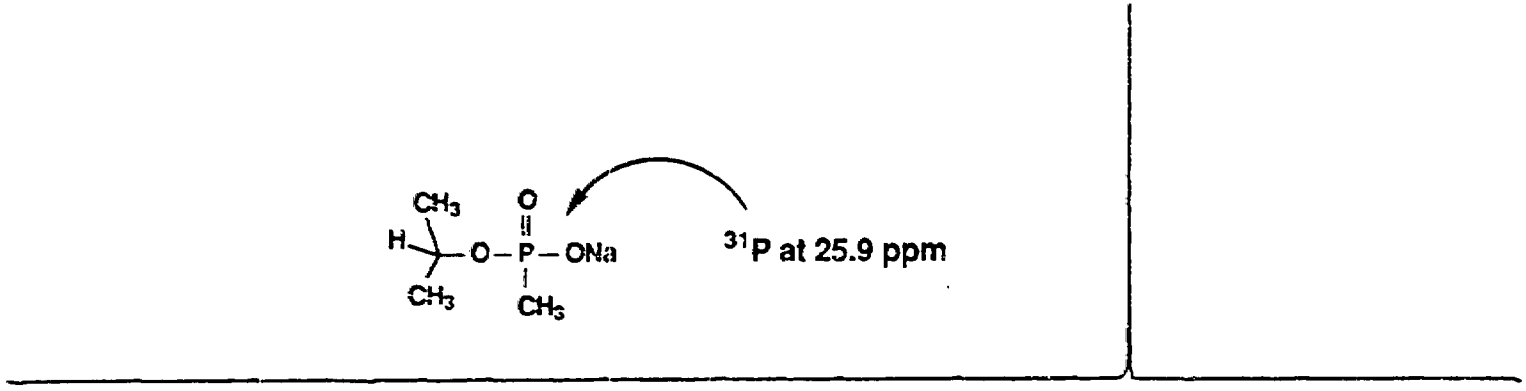


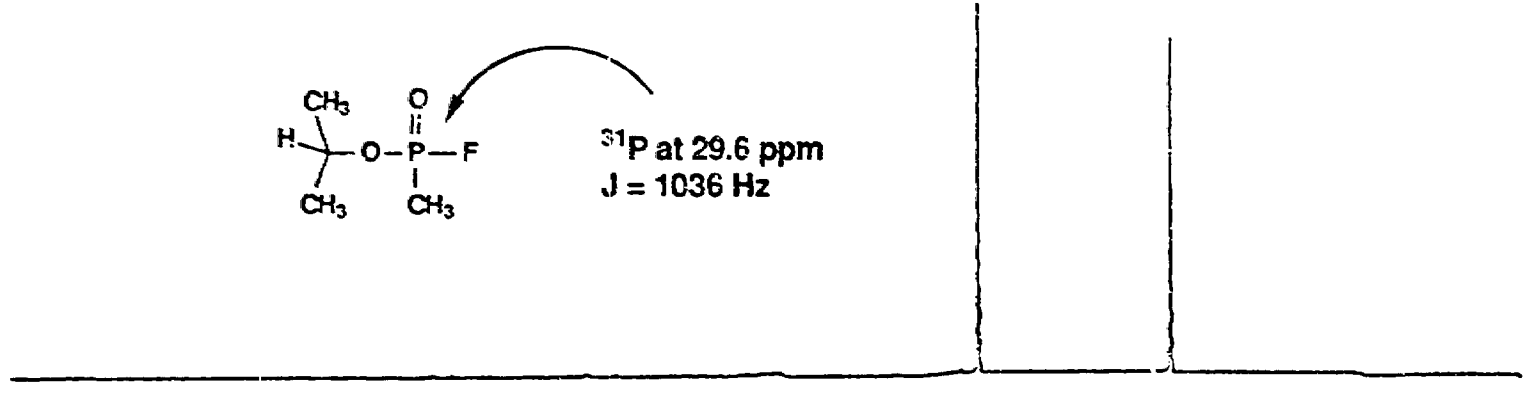
^{31}P at 29.6 ppm
J = 1036 Hz

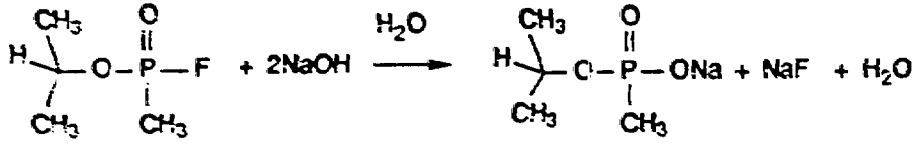
Hydrolysis Stoichiometry:



8.1.3 GB Hydrolysis in 10% Alcoholic NaOH



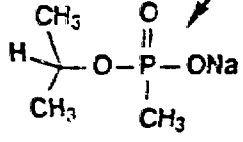




8.1.4 GB Hydrolysis in 5.25% NaOCl

Experimental Conditions: 0.02 mL of GB in 1 mL of 5.25% NaOCl :
Sample miscible with shaking.

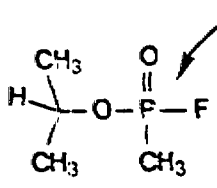
Results: Spectrum at ~ 5 min shows only:



^{31}P at 26.4 ppm

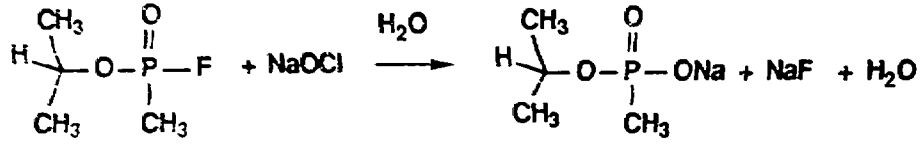
Upper Trace: Hydrolysis Product

Lower Trace: GB Starting Material

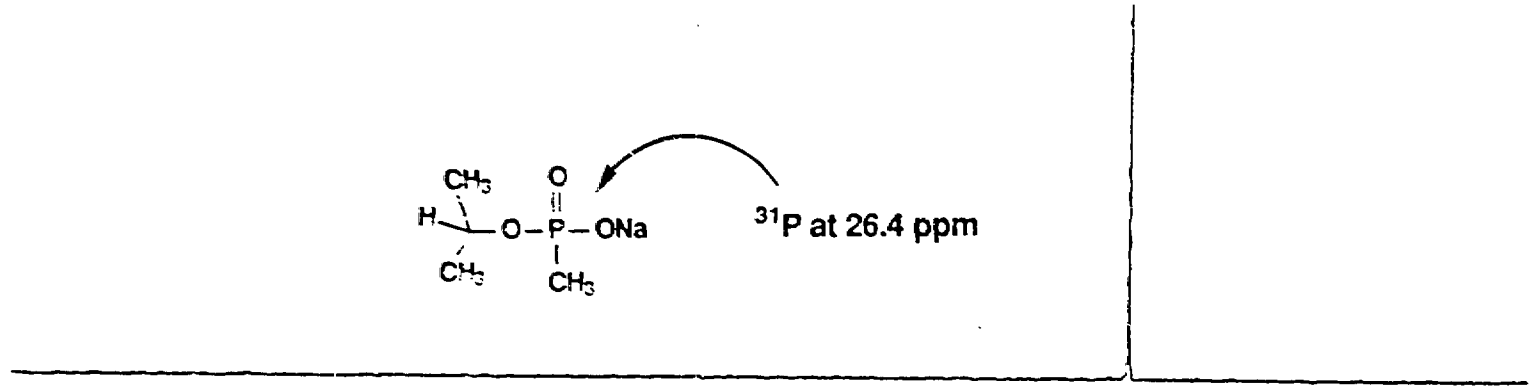


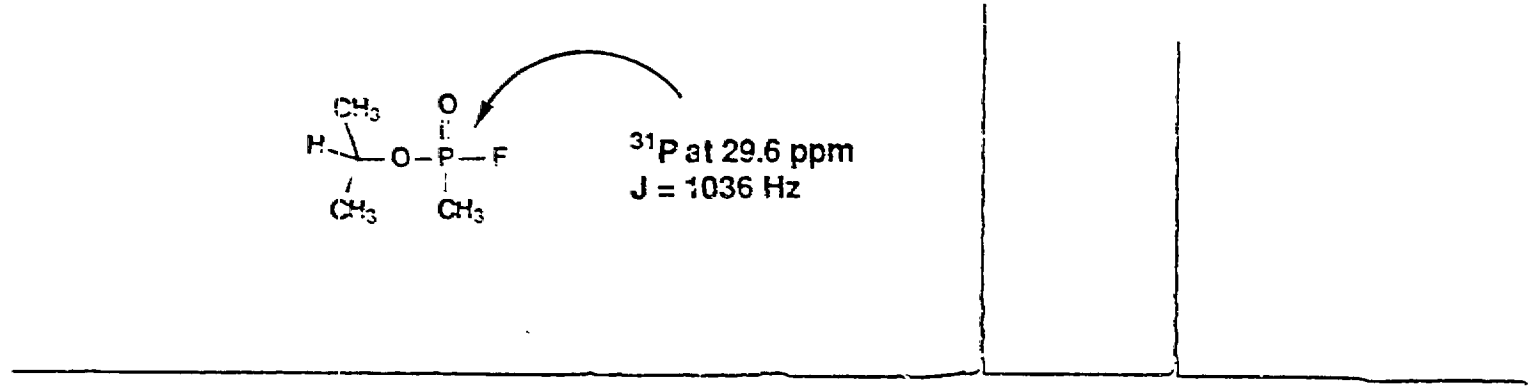
^{31}P at 29.6 ppm
J = 1036 Hz

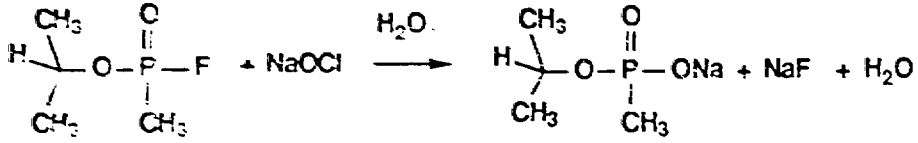
Hydrolysis Stoichiometry:



8.1.4 GB Hydrolysis in 5.25% NaOCl



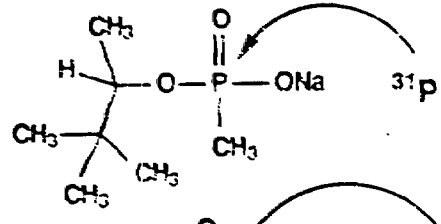




8.1.5 GD Hydrolysis in 10% NaOH

Experimental Conditions: 0.02 mL of GD in 1 mL of 10% NaOH (2.5N): pH > 14
Sample miscible with shaking.

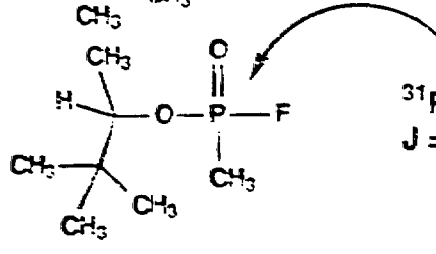
Results: Spectrum at ~ 5 min shows only:



^{31}P at 26.0 ppm

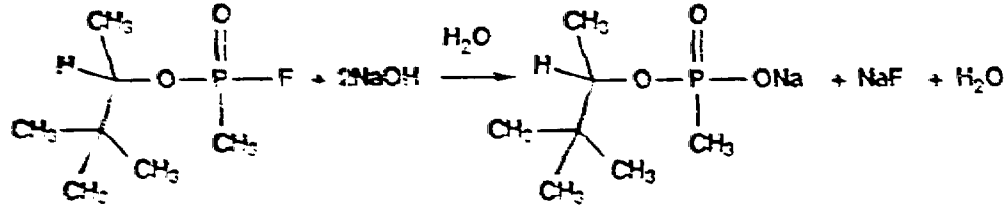
Upper Trace: Hydrolysis Product

Lower Trace: GD Starting Material

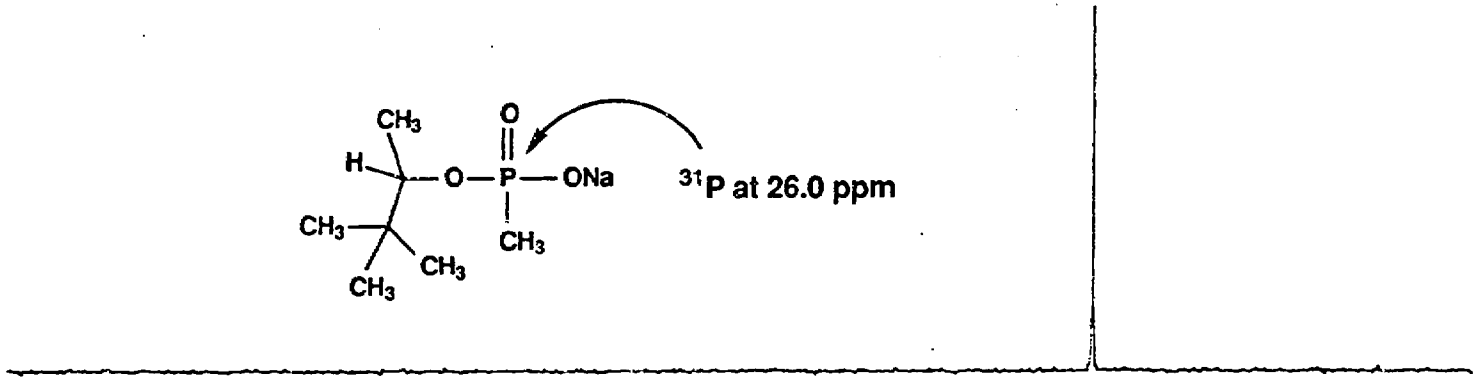


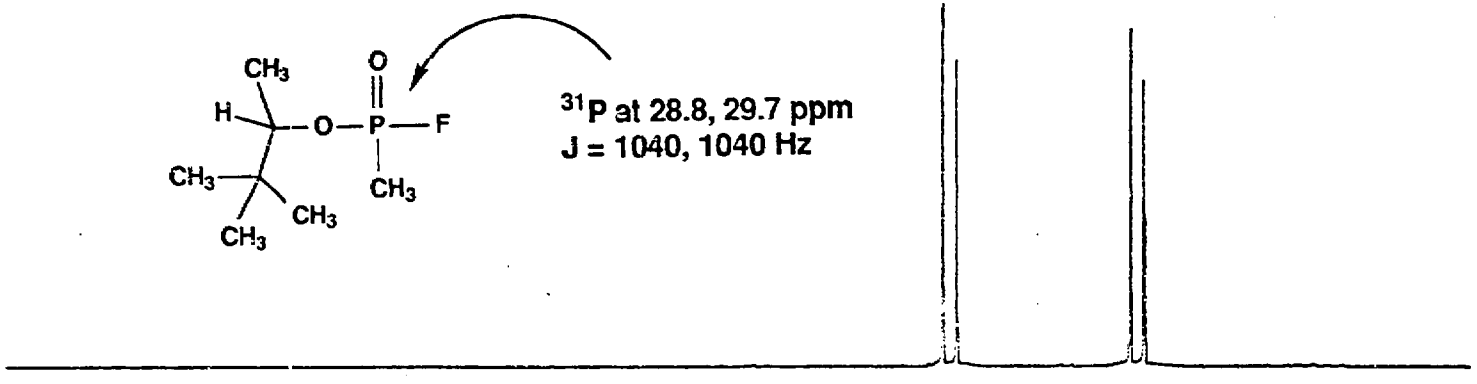
^{31}P at 28.8, 29.7 ppm
J = 1040, 1040 Hz

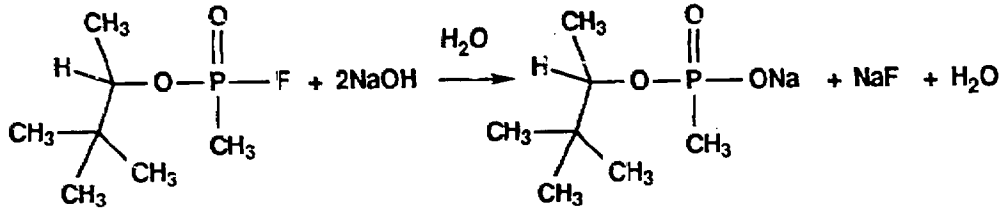
Hydrolysis Stoichiometry:



8.1.5 GD Hydrolysis in 10% NaOH



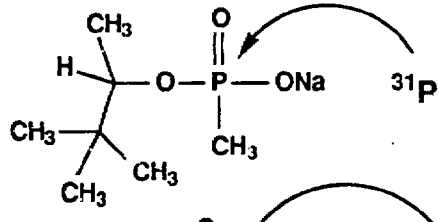




8.1.6 GD Hydrolysis in 10% Na_2CO_3

Experimental Conditions: 0.02 mL of GD in 1 mL of 10% Na_2CO_3 (2.0N): pH 12.2
Sample miscible with shaking.

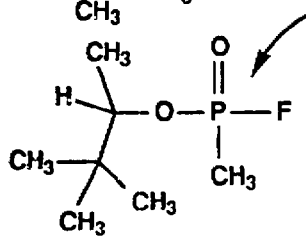
Results: Spectrum at ~ 5 min shows only:



^{31}P at 26.0 ppm

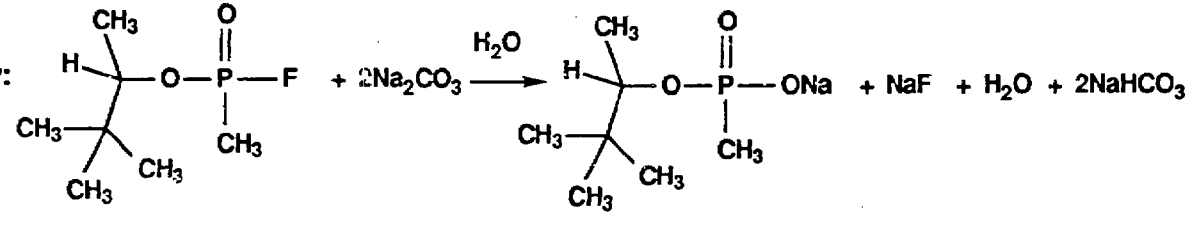
Upper Trace: Hydrolysis Product

Lower Trace: GD Starting Material

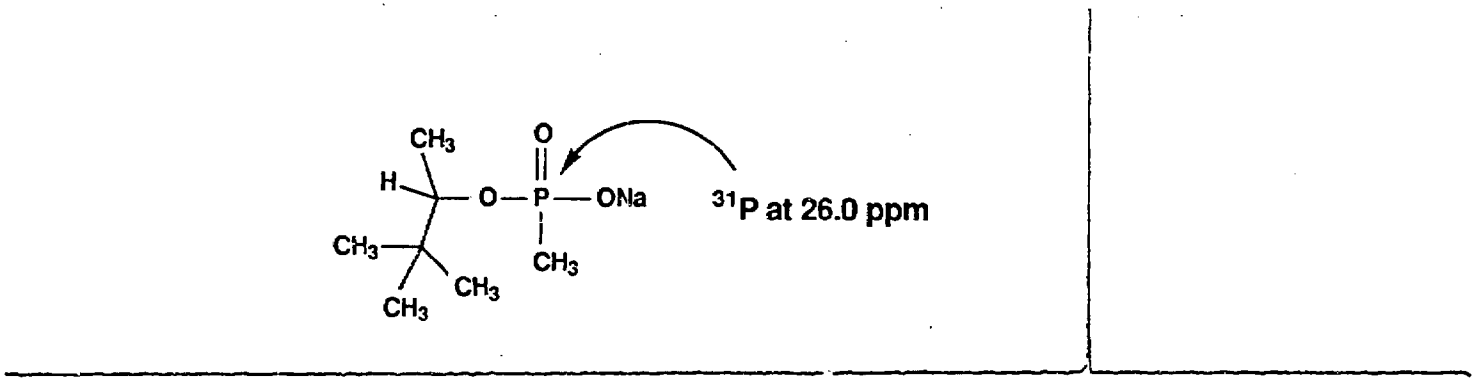


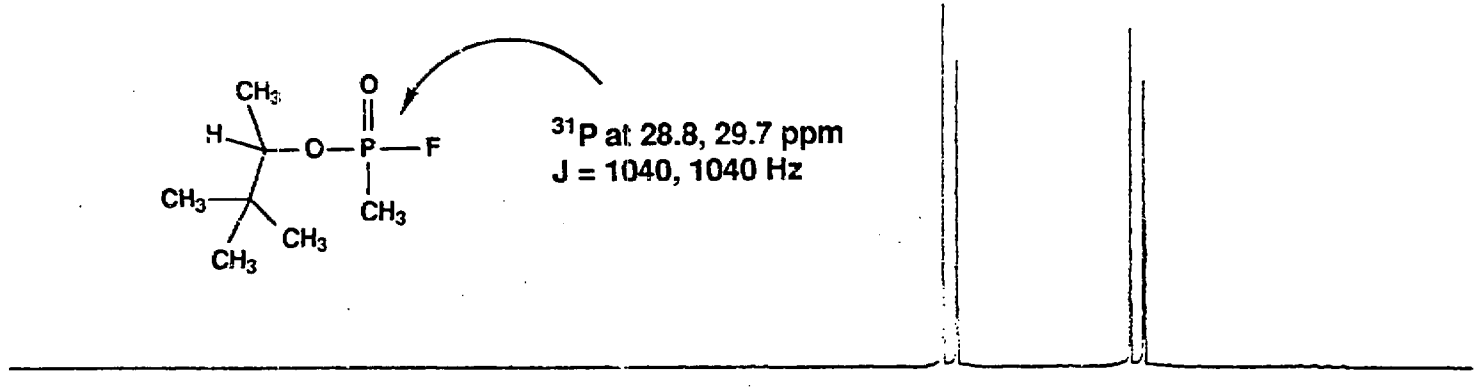
^{31}P at 28.8, 29.7 ppm
J = 1040, 1040 Hz

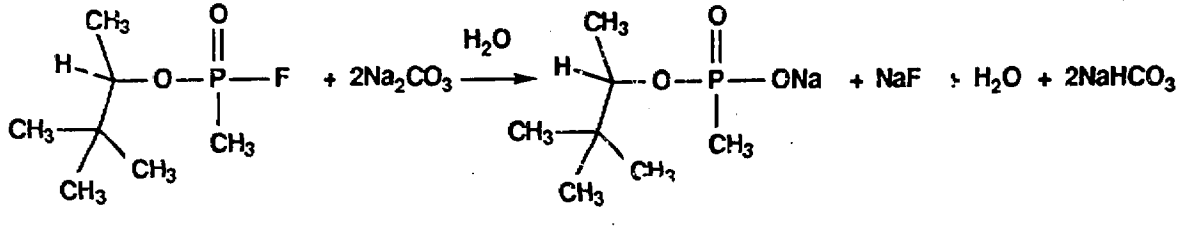
Hydrolysis Stoichiometry:



8.1.6 GD Hydrolysis in 10% Na_2CO_3



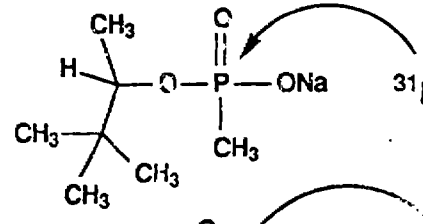




8.1.7 GD Hydrolysis in 10% Alcoholic NaOH

Experimental Conditions: 0.02 mL of GD in 1 mL of 10% Alcoholic NaOH (2.5N): pH > 14
Sample miscible with shaking.

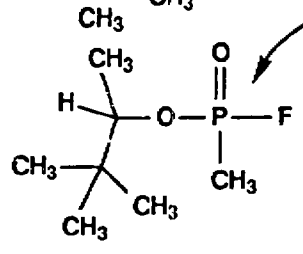
Results: Spectrum at ~ 5 min shows only:



^{31}P at 25.6 ppm

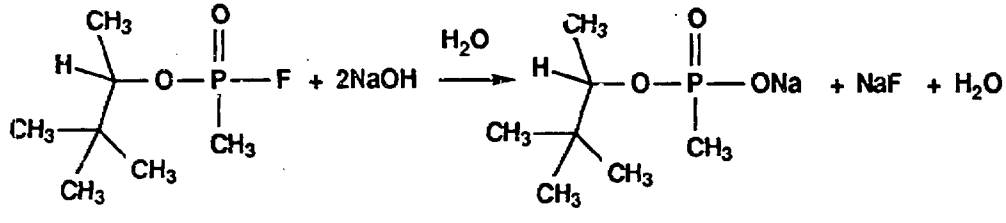
Upper Trace: Hydrolysis Product

Lower Trace: GD Starting Material

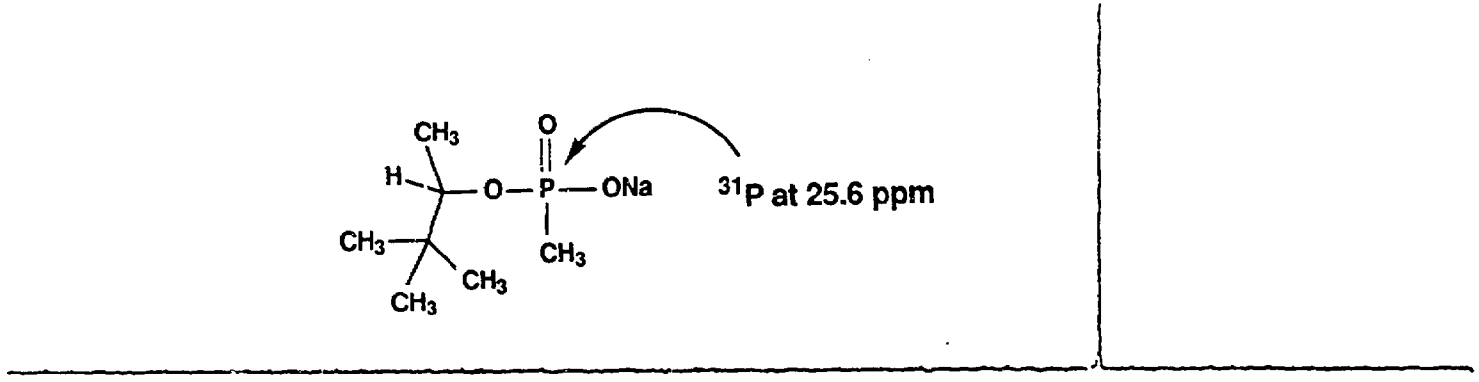


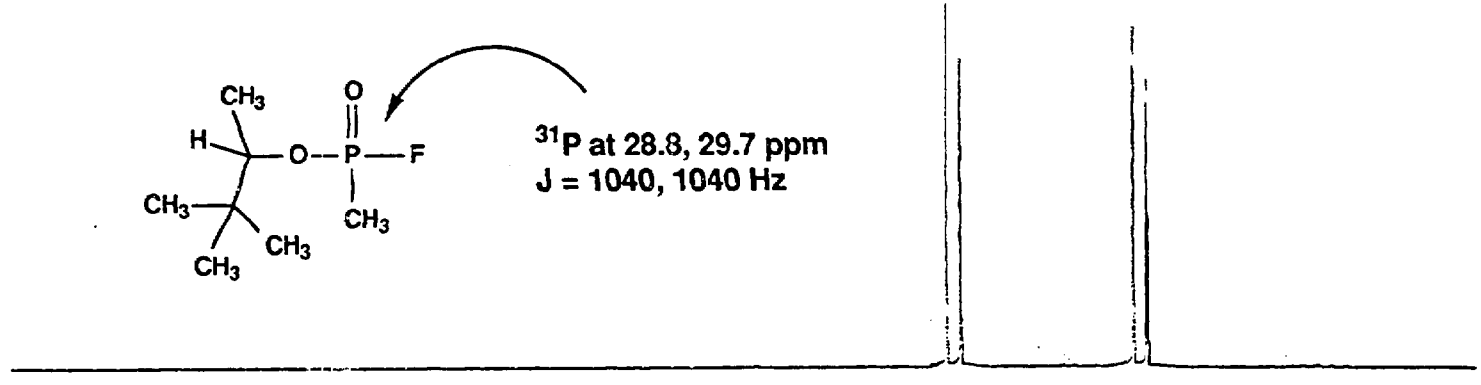
^{31}P at 28.8, 29.7 ppm
J = 1040, 1040 Hz

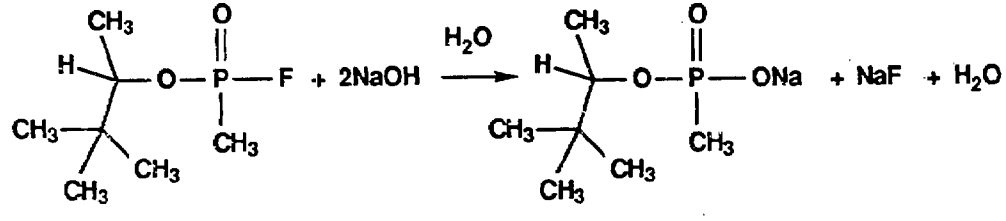
Hydrolysis Stoichiometry:



8.1.7 GD Hydrolysis in 10% Alcoholic NaOH



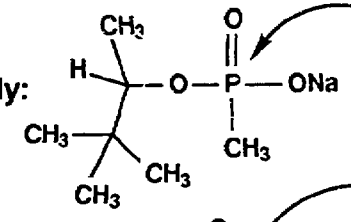




8.1.8 GD Hydrolysis in 5.25% NaOCl

Experimental Conditions: 0.02 mL of GD in 1 mL of 5.25% NaOCl:
Sample miscible with shaking.

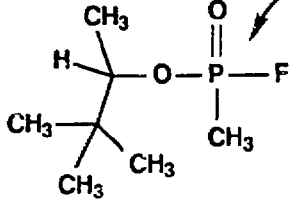
Results: Spectrum at ~ 10 min shows only:



^{31}P at 26.2 ppm

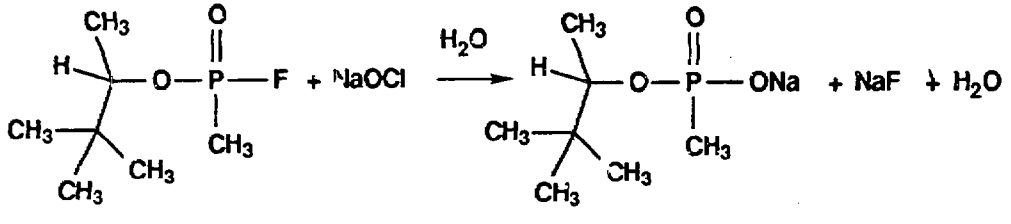
Upper Trace: Hydrolysis Product

Lower Trace: GD Starting Material

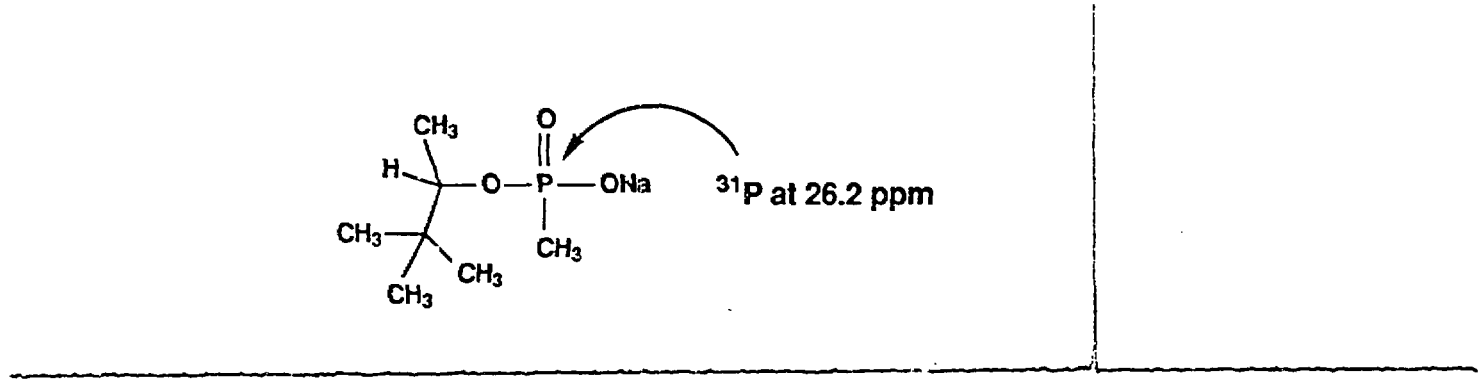


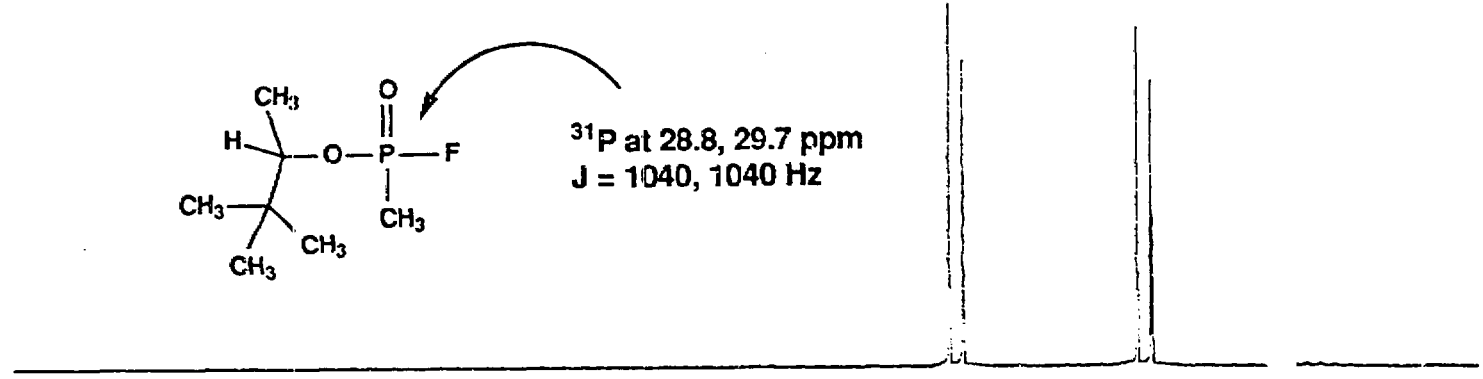
^{31}P at 28.8, 29.7 ppm
J = 1040, 1040 Hz

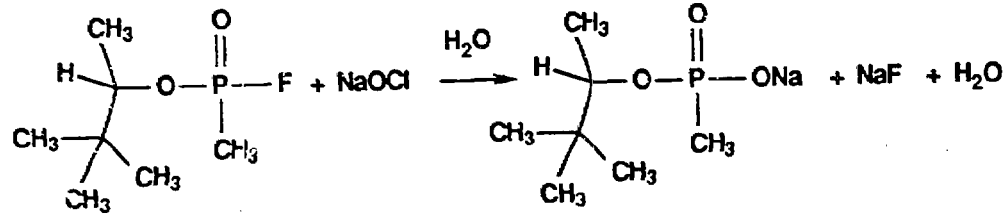
Hydrolysis Stoichiometry:



8.1.8 GD Hydrolysis in 5.25% NaOCl



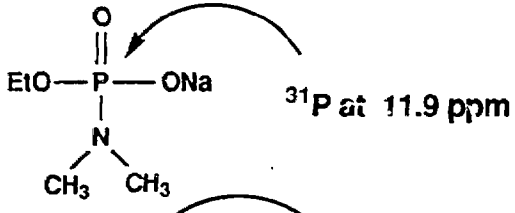




8.1.9 GA Hydrolysis in 10% NaOH

Experimental Conditions: 0.02 mL of GA in 1 mL of 10% NaOH (2.5N): pH > 14
Sample miscible with shaking.

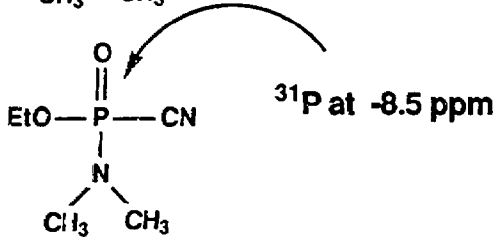
Results: Spectrum at ~ 30 min shows only:



^{31}P at 11.9 ppm

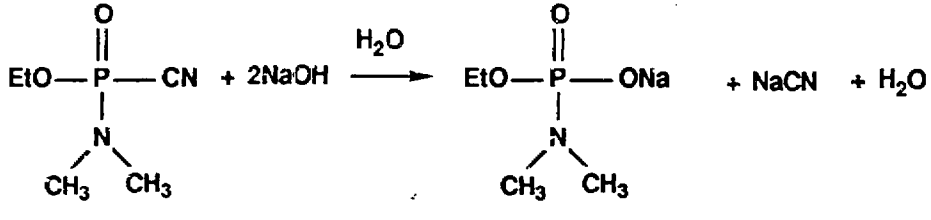
Upper Trace: Hydrolysis Product

Lower Trace: GA Starting Material

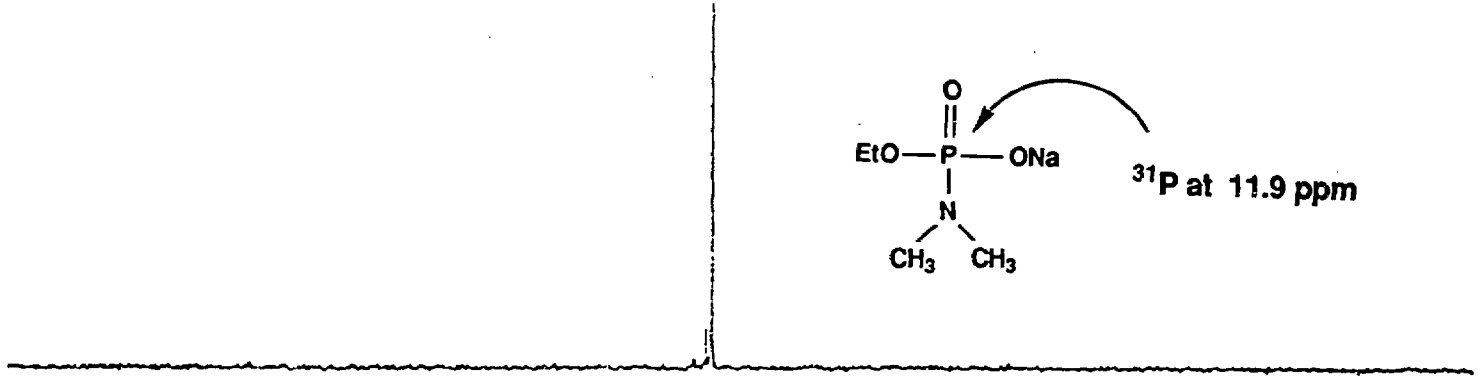


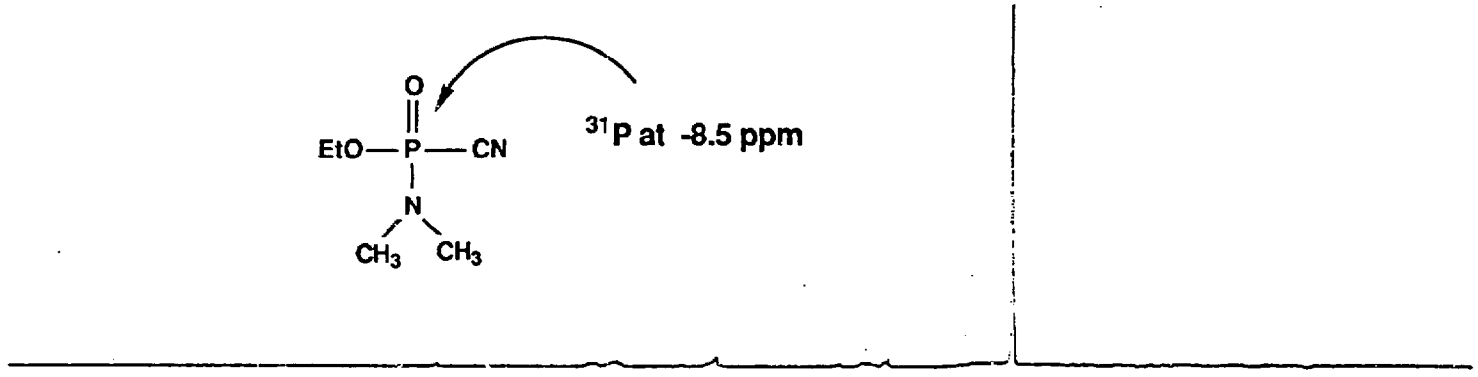
^{31}P at -8.5 ppm

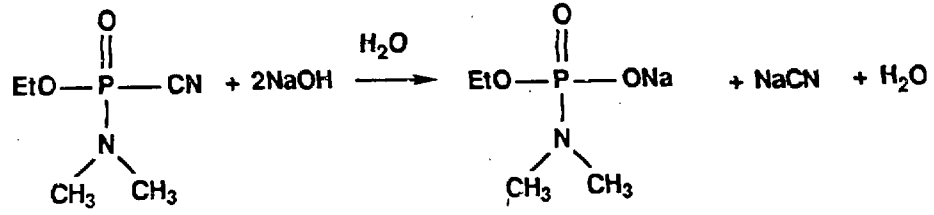
Hydrolysis Stoichiometry:



8.1.9 GA Hydrolysis in 10% NaOH



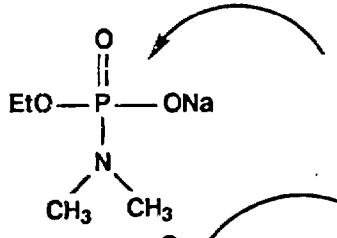




8.1.10 GA Hydrolysis in 10% Na_2CO_3

Experimental Conditions: 0.02 mL of GD in 1 mL of 10% Na_2CO_3 (2.0N): pH 12.2
Sample miscible with shaking.

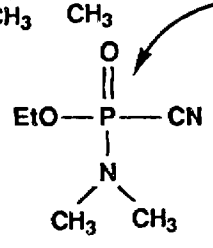
Results: Spectrum at ~ 5 min shows only:



^{31}P at 12.0 ppm

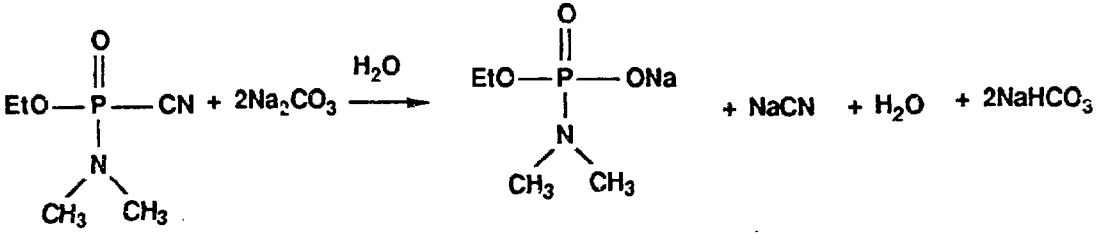
Upper Trace: Hydrolysis Product

Lower Trace: GA Starting Material

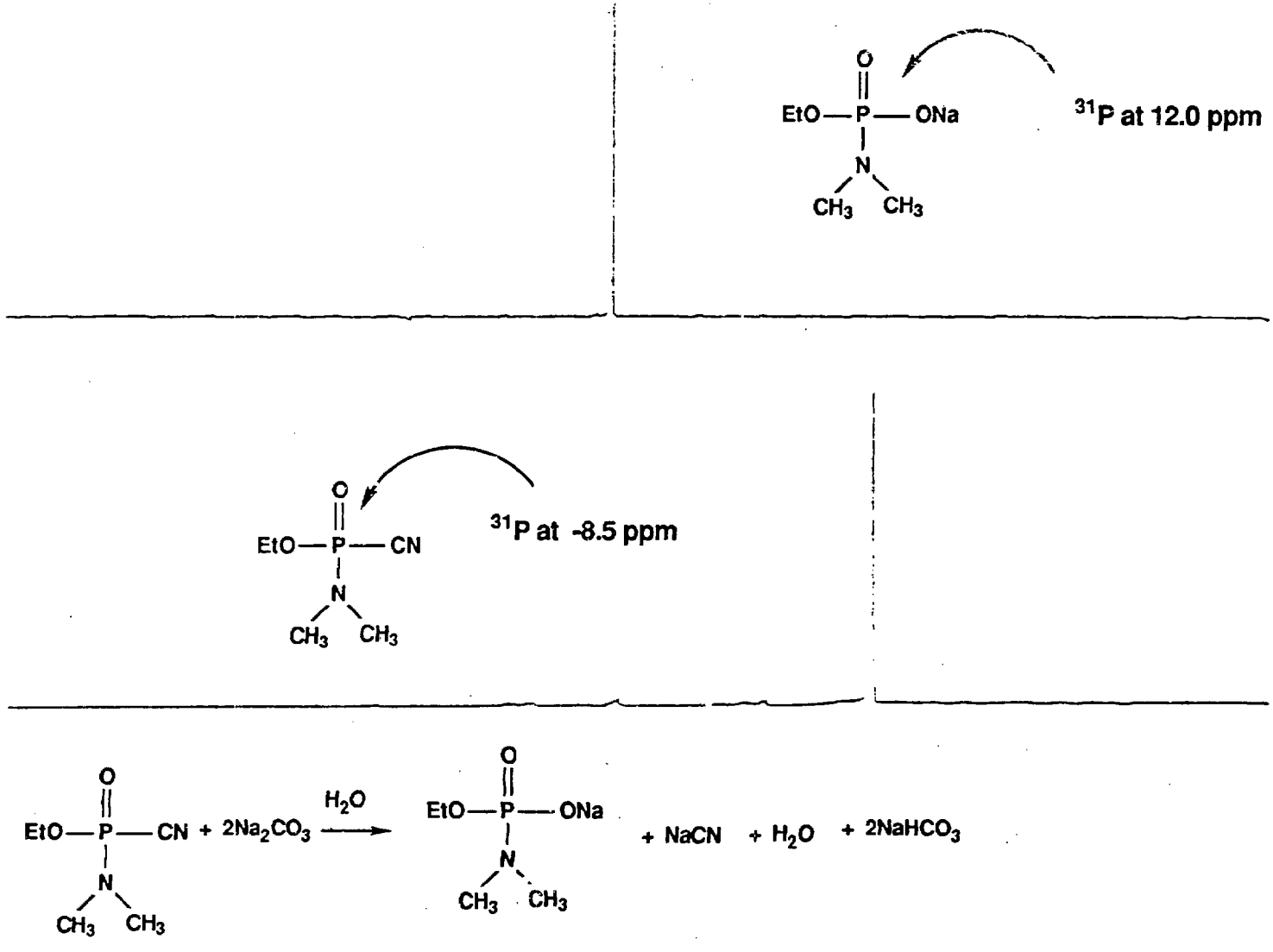


^{31}P at -8.5 ppm

Hydrolysis Stoichiometry:



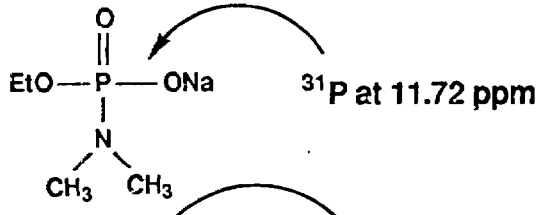
8.1.10 GA Hydrolysis in 10% Na_2CO_3



8.1.11 GA Hydrolysis in 10% Alcoholic NaOH

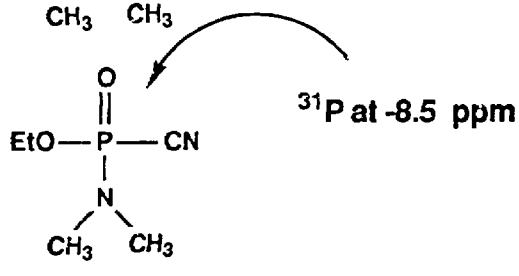
Experimental Conditions: 0.02 mL of GA in 1 mL of 10% Alcoholic NaOH (2.5N): pH > 14
Sample miscible with shaking.

Results: Spectrum at ~ 30 min shows only:

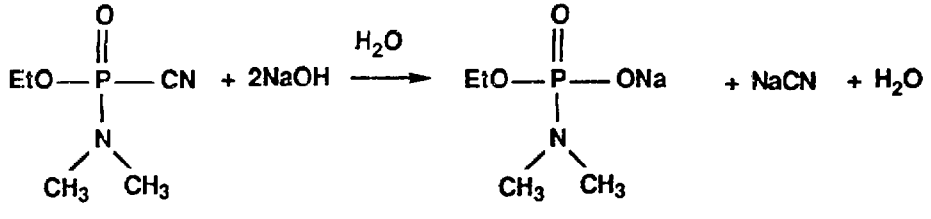


Upper Trace: Hydrolysis Product

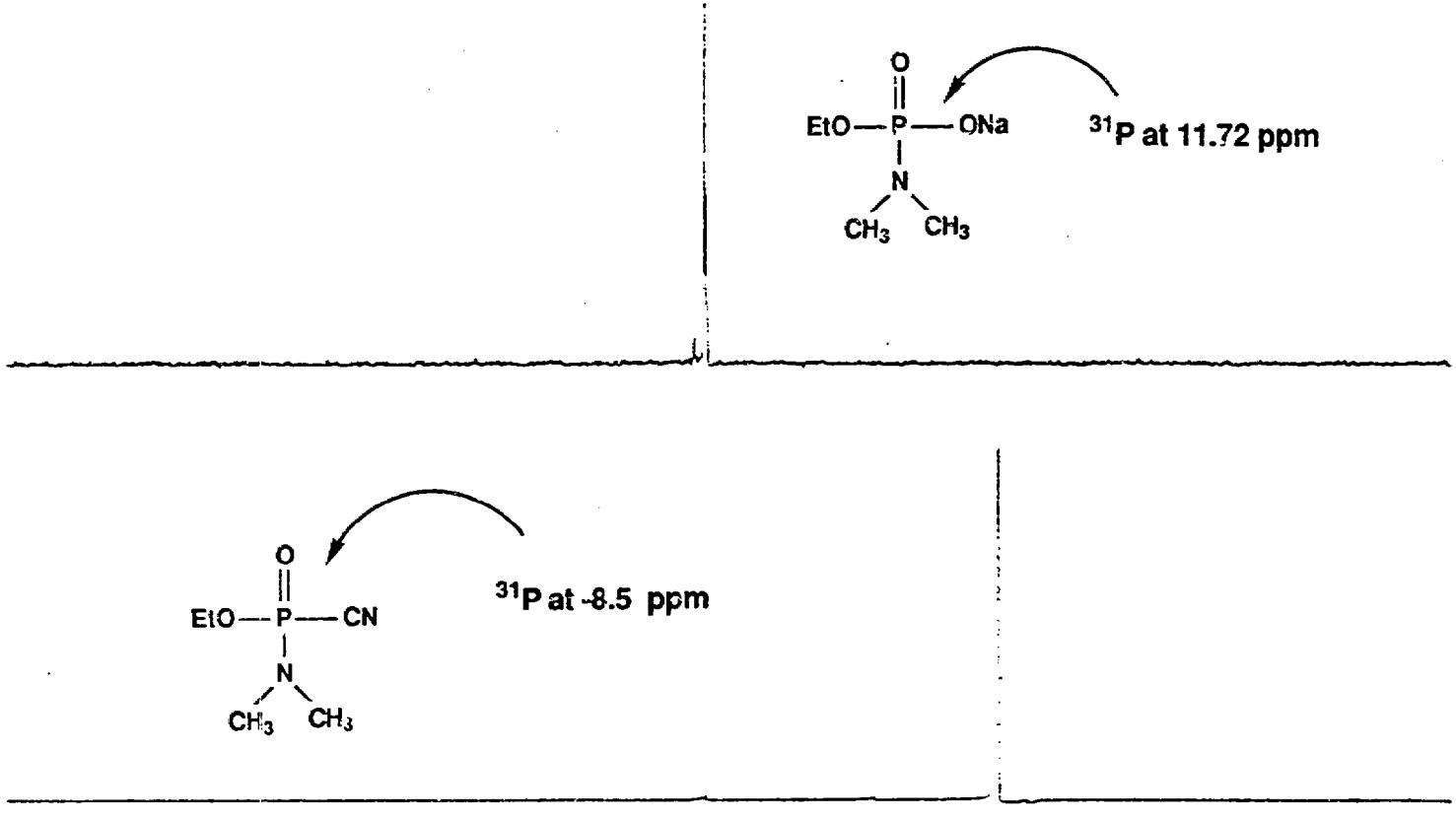
Lower Trace: GA Starting Material

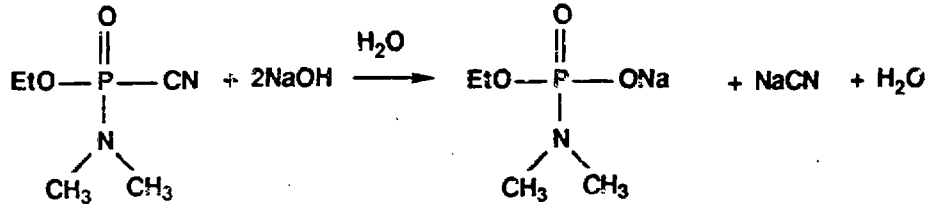


Hydrolysis Stoichiometry:



8.1.11 GA Hydrolysis in 10% Alcoholic NaOH

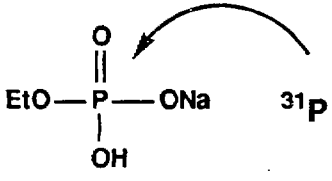




8.1.12 GA Hydrolysis in 5.25% NaOCl

Experimental Conditions: 0.02 mL of GA in 1 mL of 5.25% NaOCl:
Sample miscible with shaking.

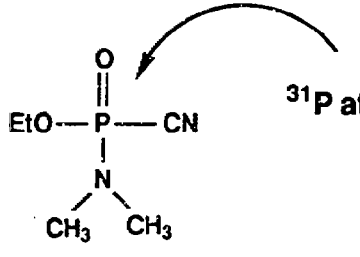
Results: Spectrum at ~ 5 min shows only:



^{31}P at 4.9 ppm

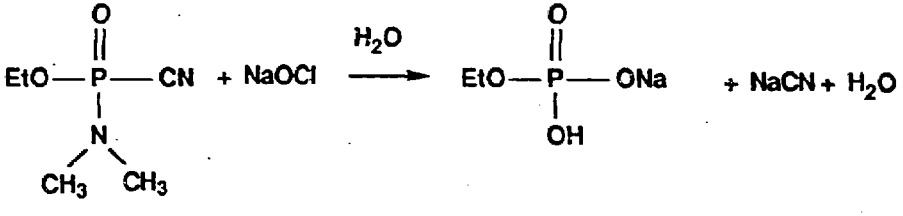
Upper Trace: Hydrolysis Product

Lower Trace: GA Starting Material

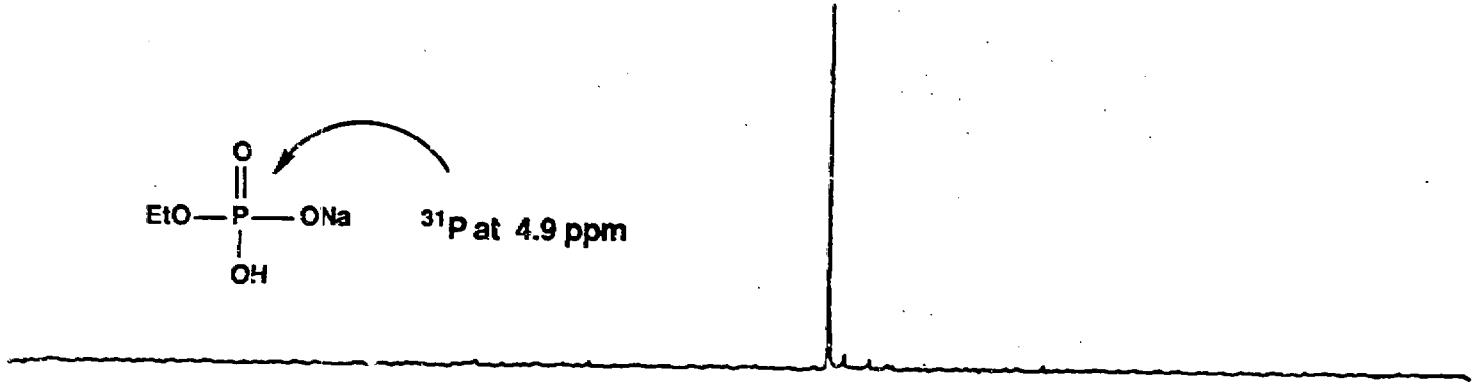


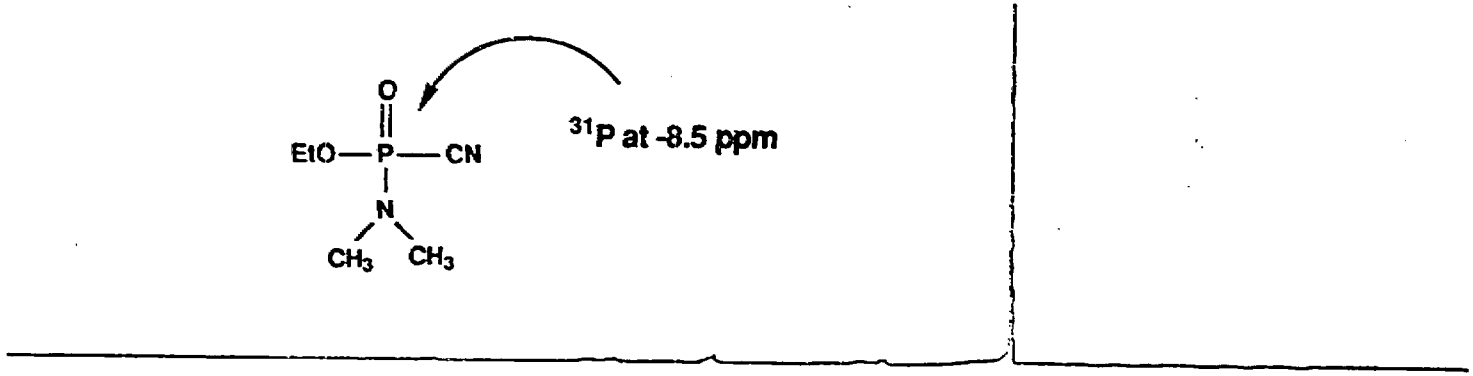
^{31}P at -8.5 ppm

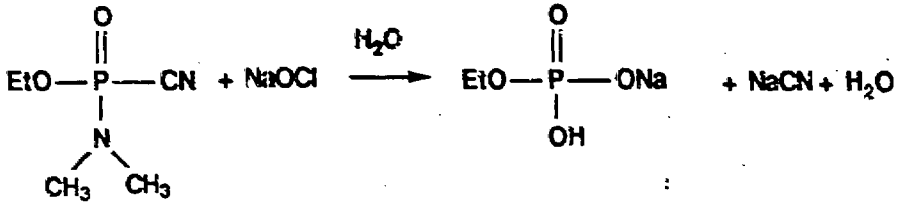
Hydrolysis Stoichiometry:



8.1.12 GA Hydrolysis in 5.25% NaOCl



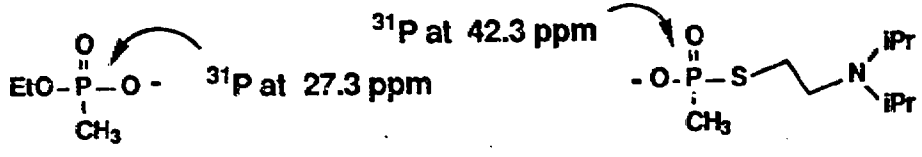




8.1.13 VX Hydrolysis in 10% Alcoholic NaOH

Experimental Conditions: 0.02 mL of VX in 1.5 mL of 10% Alcoholic NaOH (2.5N): pH > 14
Sample miscible with shaking.

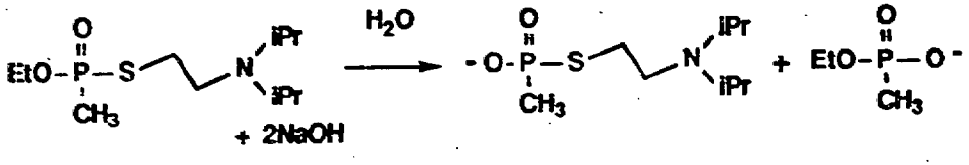
Results: Spectrum at ~ 25 min shows:



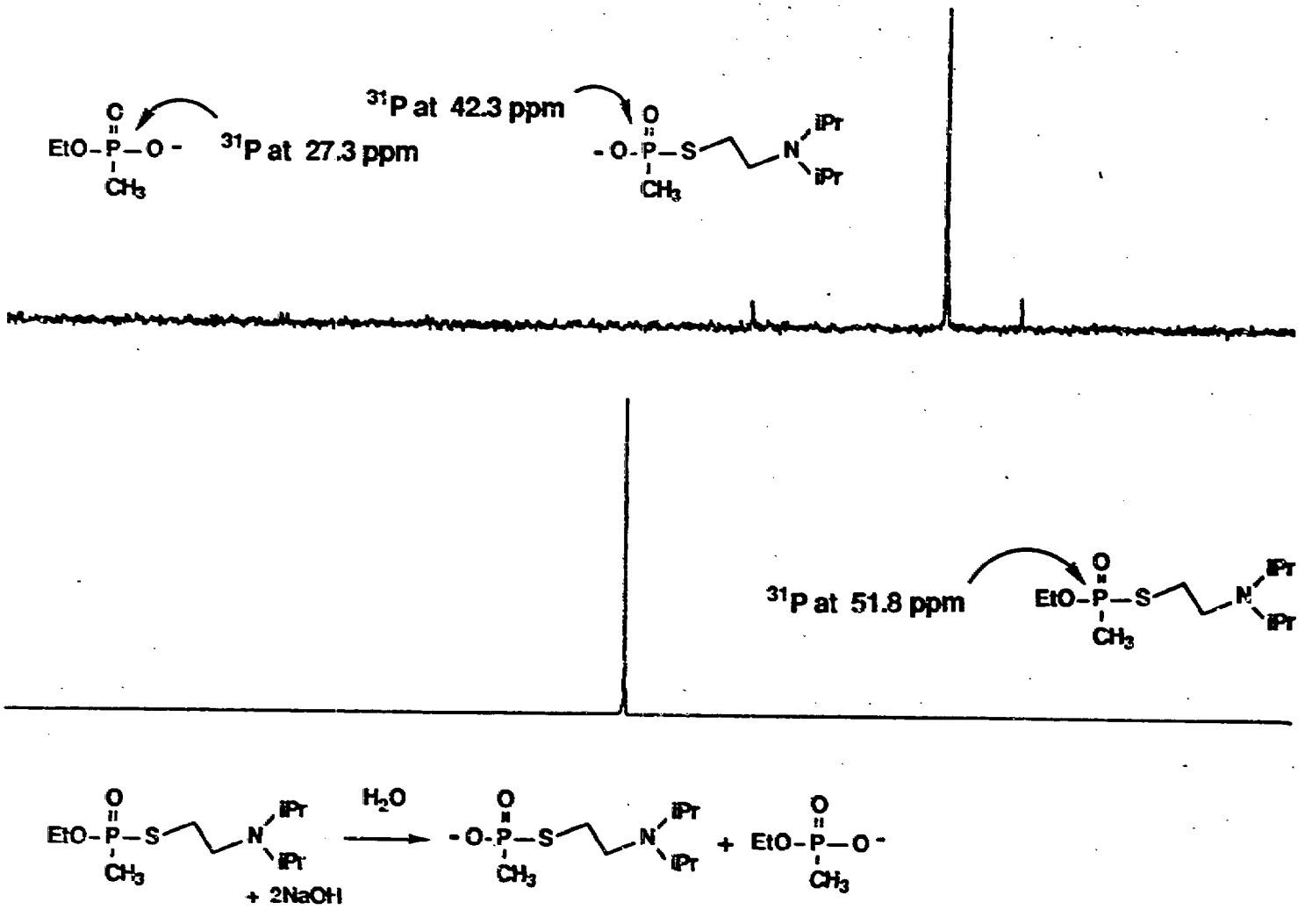
Upper Trace: Hydrolysis Product

Lower Trace: VX Starting Material

Hydrolysis Stoichiometry:



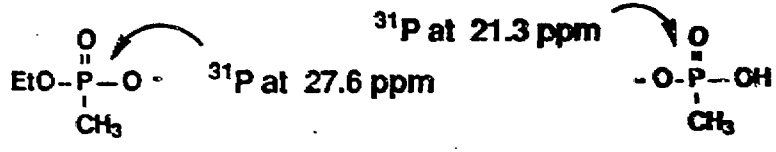
8.1.13 VX Hydrolysis in 10% Alcoholic NaOH



8.1.14 VX Hydrolysis in 5.25% NaOCl

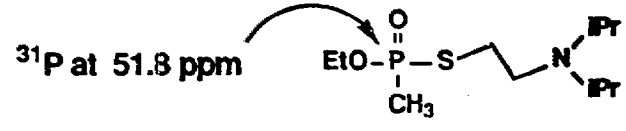
Experimental Conditions: 0.02 mL of VX in 1.0 mL of 5.25% NaOCl : Sample miscible with shaking.

Results: Spectrum at ~ 18 Hr shows:

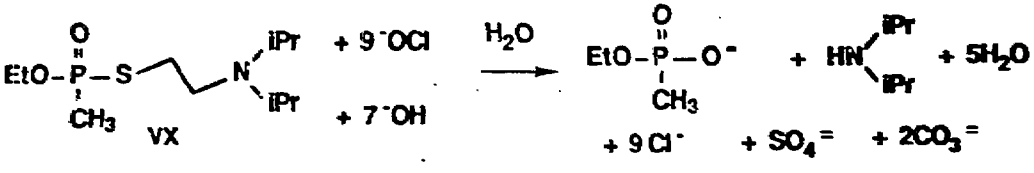


Upper Trace: Hydrolysis Product

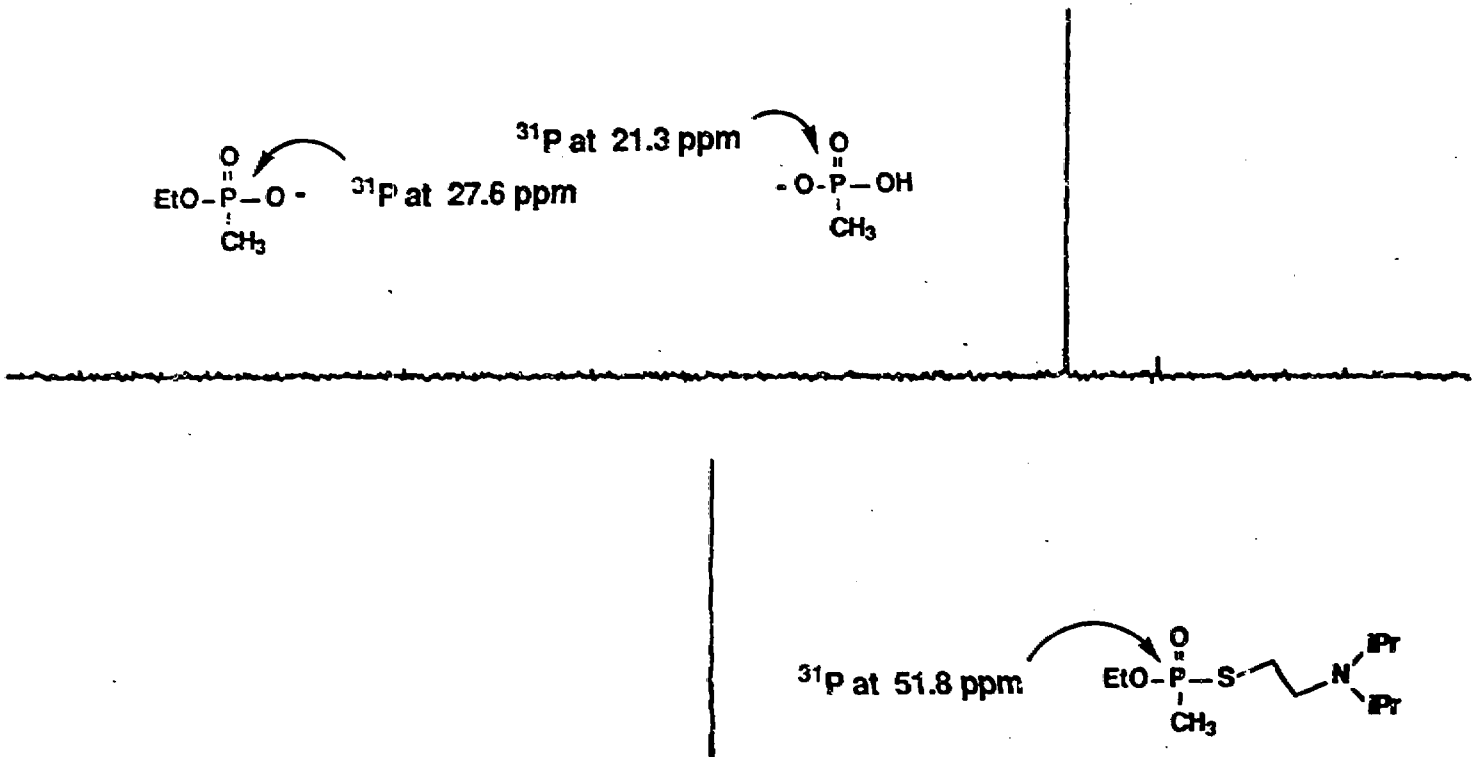
Lower Trace: VX Starting Material

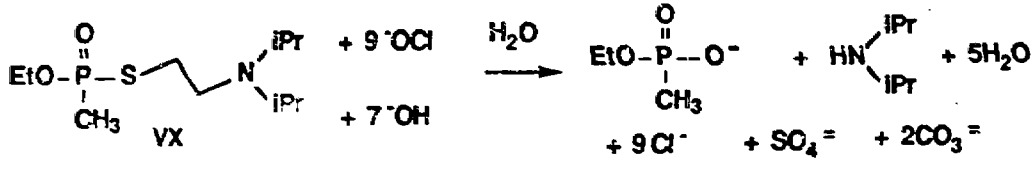


Hydrolysis Stoichiometry:



8.1.14 VX Hydrolysis in 5.25% NaOCl

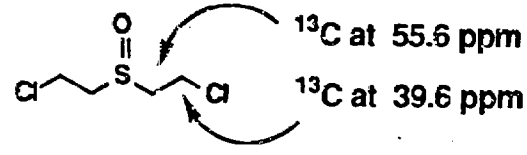




8.1.15 HD Oxidation in Conc. HNO_3

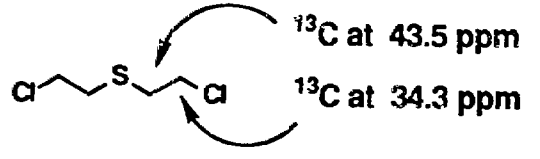
Experimental Conditions: 0.02 mL of HD in 1 mL of Conc. HNO_3:
Sample miscible with shaking.

Results: Spectrum shows only:

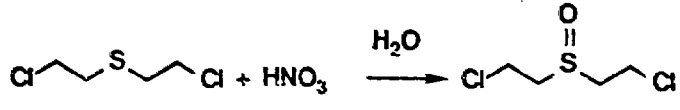


Upper Trace: Oxidation Product

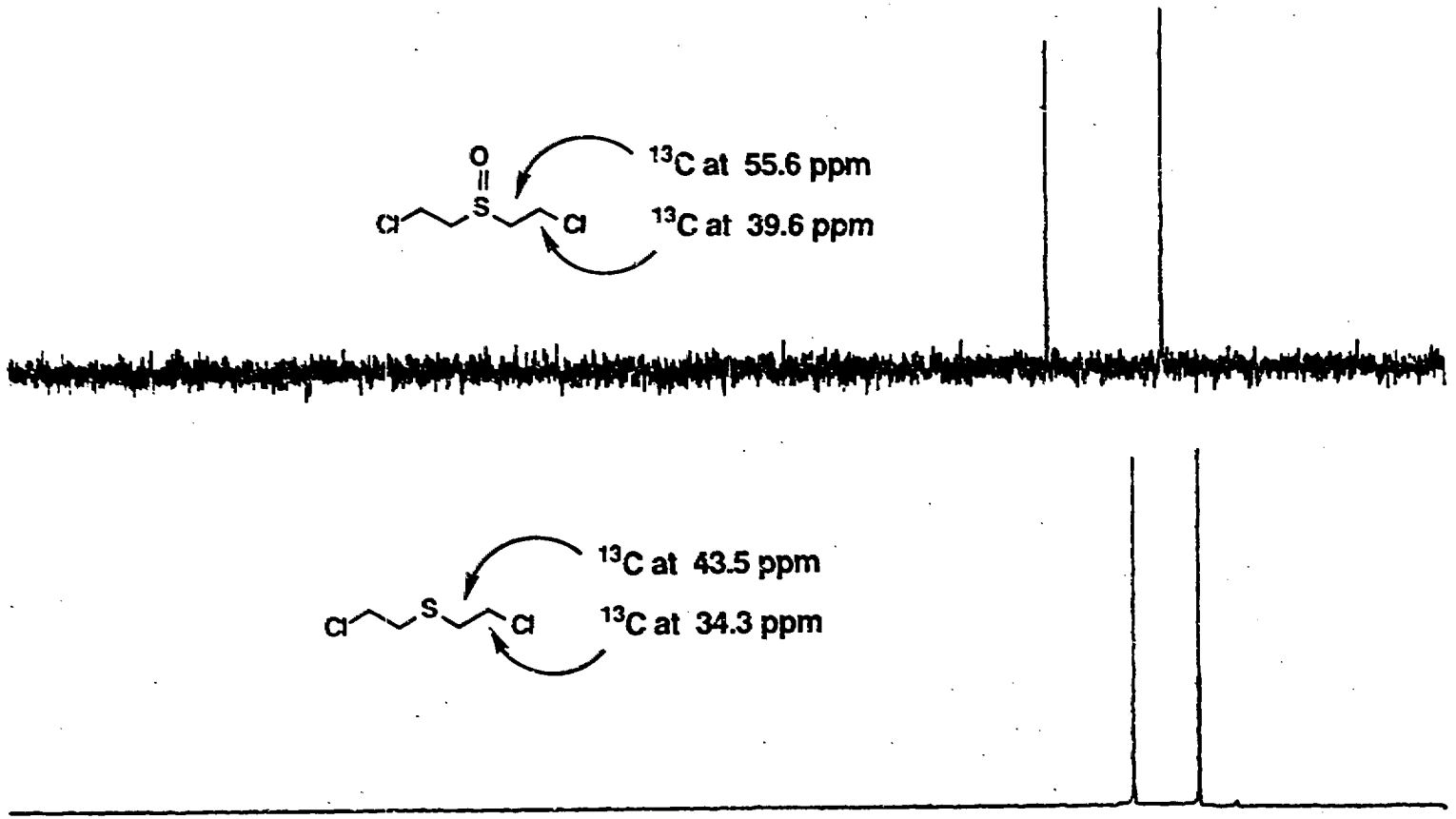
Lower Trace: HD Starting Material



Hydrolysis Stoichiometry:



8.1.15 HD Oxidation in Conc. HNO_3



Hydrolysis Stoichiometry: Cl–CH$_2$–CH$_2$–S–CH$_2$–CH$_2$–Cl + HNO_3 $\xrightarrow{H_2O}$ Cl–CH$_2$–CH$_2$–S(=O)–CH$_2$–CH$_2$–Cl

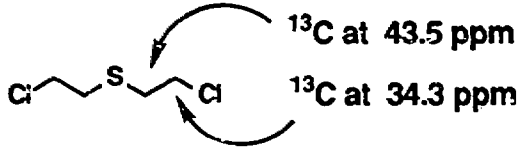
8.1.16 HD Oxidation in 5.25% NaOCl

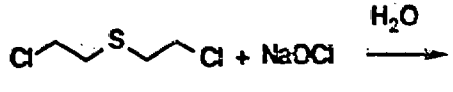
Experimental Conditions: 0.02 mL of HD in 1 mL of 5.25% NaOCl: Sample miscible with shaking.

Results: Spectrum shows multiple oxidation products - no HD present:

Upper Trace: Oxidation Product

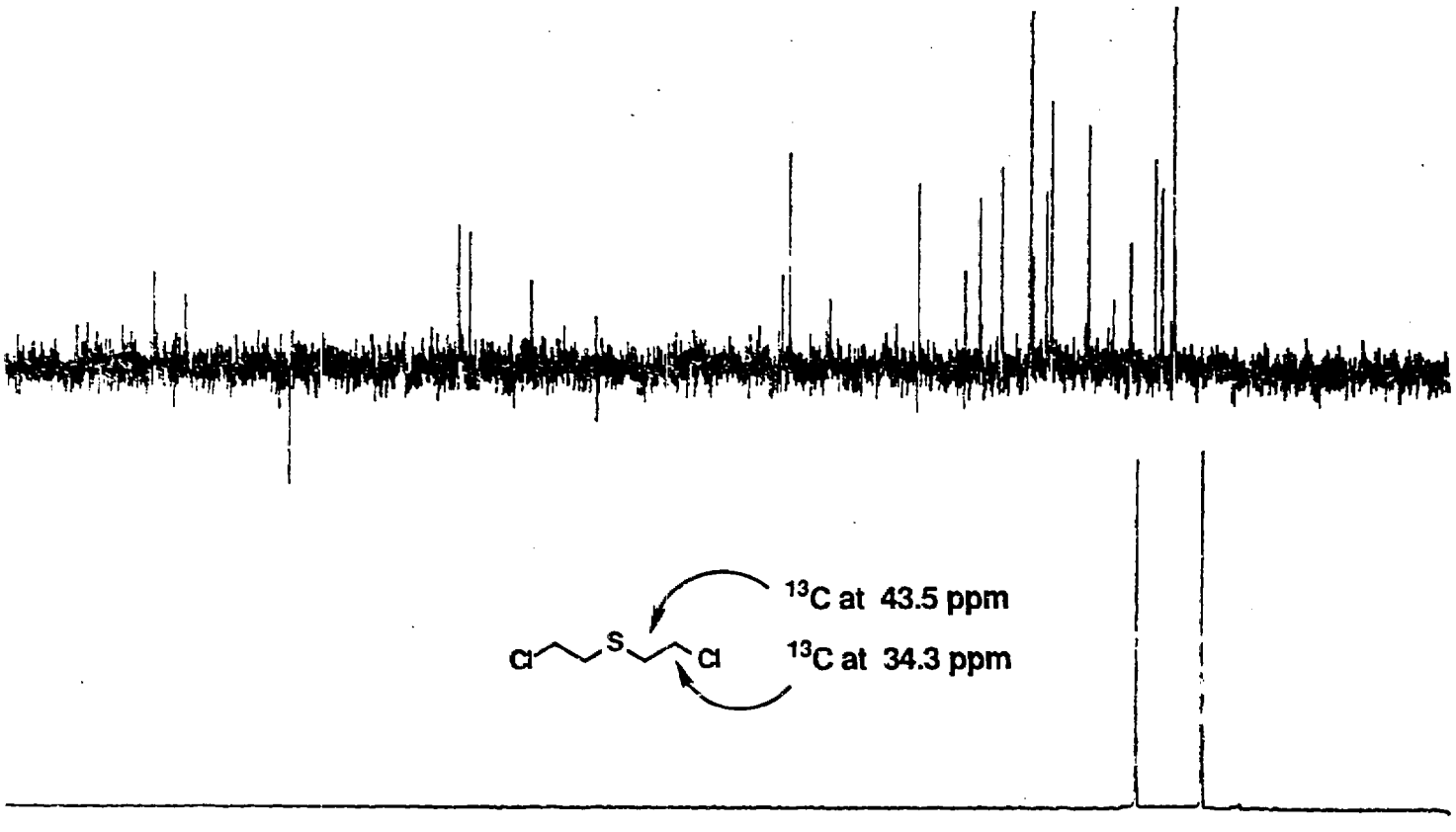
Lower Trace: HD Starting Material

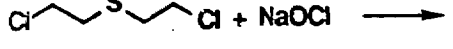


Hydrolysis Stoichiometry: 

Multiple Oxidation Products

8.1.16 HD Oxidation in 5.25% NaOCl



Hydrolysis Stoichiometry:  + NaOCl $\xrightarrow{H_2O}$ Multiple Oxidation Products

8.1.17 Lewisite Hydrolysis in 5.25% NaOCl

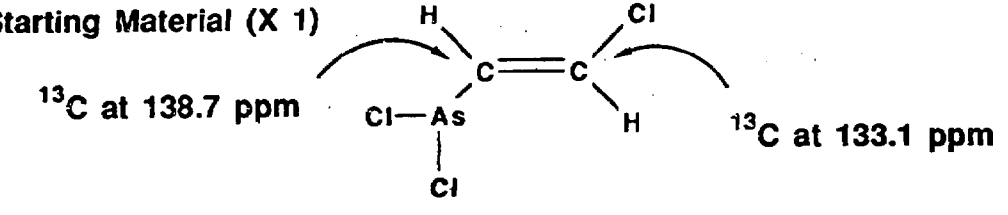
Experimental Conditions: 0.02 mL of Lewisite in 1.0 mL of 5.25% NaOCl : Sample miscible with shaking; a lot of gas (e.g., acetylene) evolved (spectrum amplitude of product (middle trace) increased X100 (top trace) to show trace peaks).

Results: Spectrum at 10 min shows: No Lewisite present; virtually no signal at all. Sample appears to have reacted to give acetylene (gas).

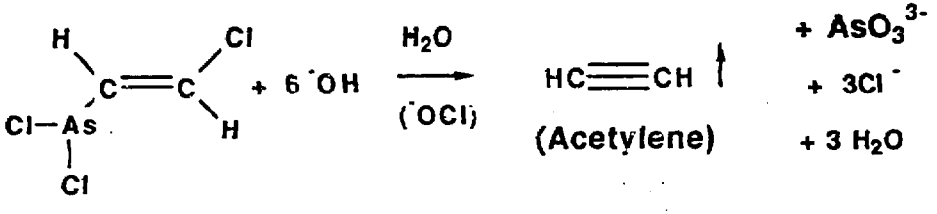
Upper Trace: Hydrolysis Product (X 100)
Middle Trace: Hydrolysis Product (X 1)

No Carbon: Thus no spectral lines.

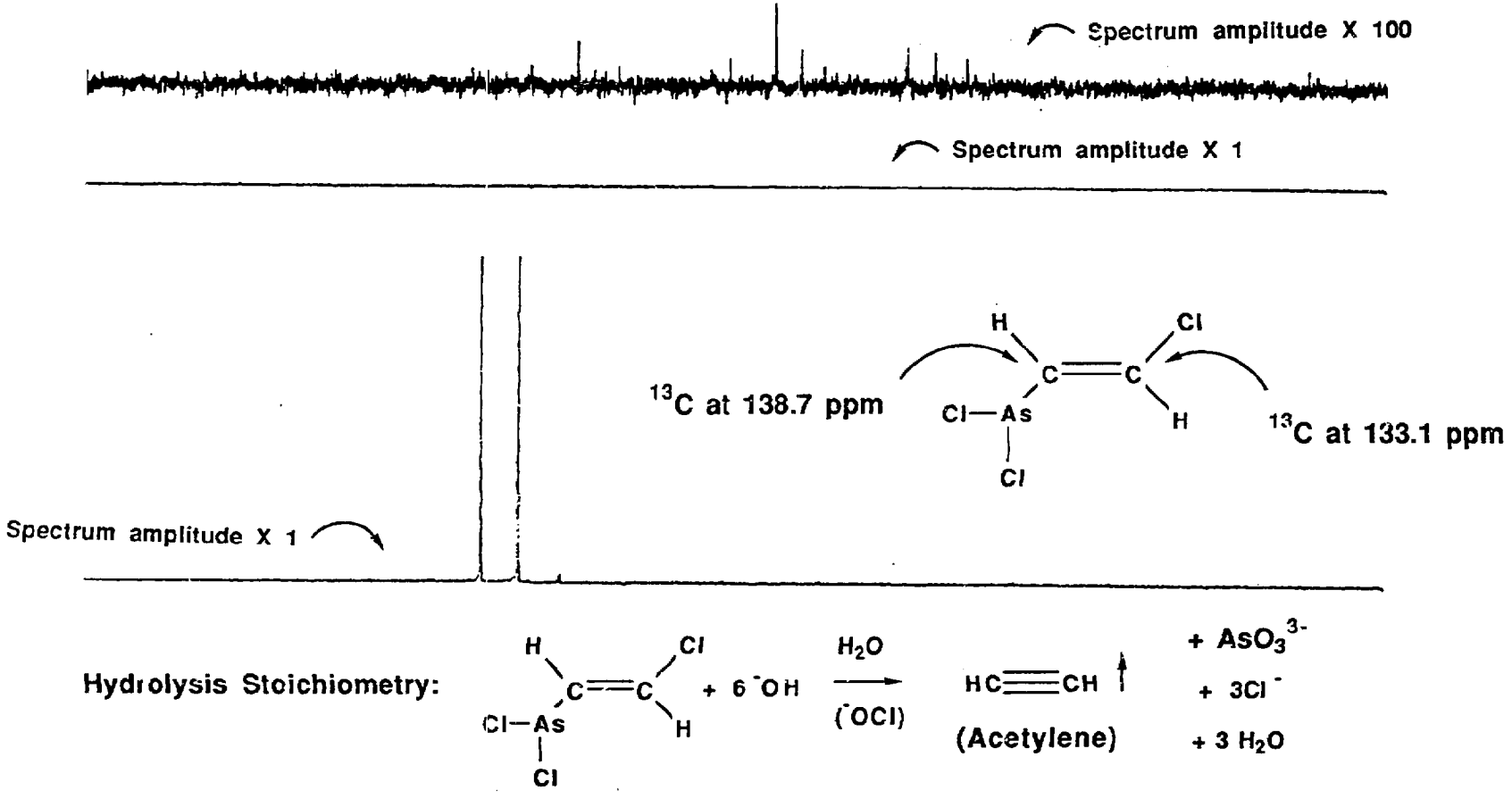
Lower Trace: Lewisite Starting Material (X 1)



Hydrolysis Stoichiometry:



8.1.17 Lewisite Hydrolysis in 5.25% NaOCl



8.1.18 Lewisite Hydrolysis in 10% Na_2CO_3

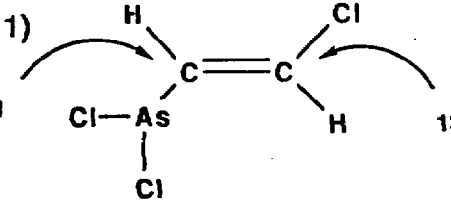
Experimental Conditions: 0.02 mL of Lewisite in 0.2 mL isopropanol plus 1.0 mL of 10% Na_2CO_3 : Sample miscible; a lot of gas (e.g., acetylene) evolved. Major peak at δ167.3 may be acetylene in solution; there are trace unknown peaks at δ161.7, 144.7, 132.6 (spectrum amplitude increased X10 to show these trace peaks).

Results: Spectrum at 10 min shows: No Lewisite present; ^{13}C signal due to solvent. Sample appears to have reacted to give acetylene (gas) in solution.

Upper Trace: Hydrolysis Product (X 10)
Middle Trace: Hydrolysis Product (X 1)

No Lewisite carbons, peaks due to solvent (isopropanol).

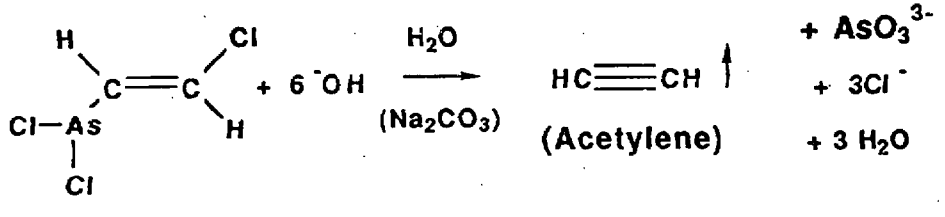
Lower Trace: Lewisite Starting Material (X 1)



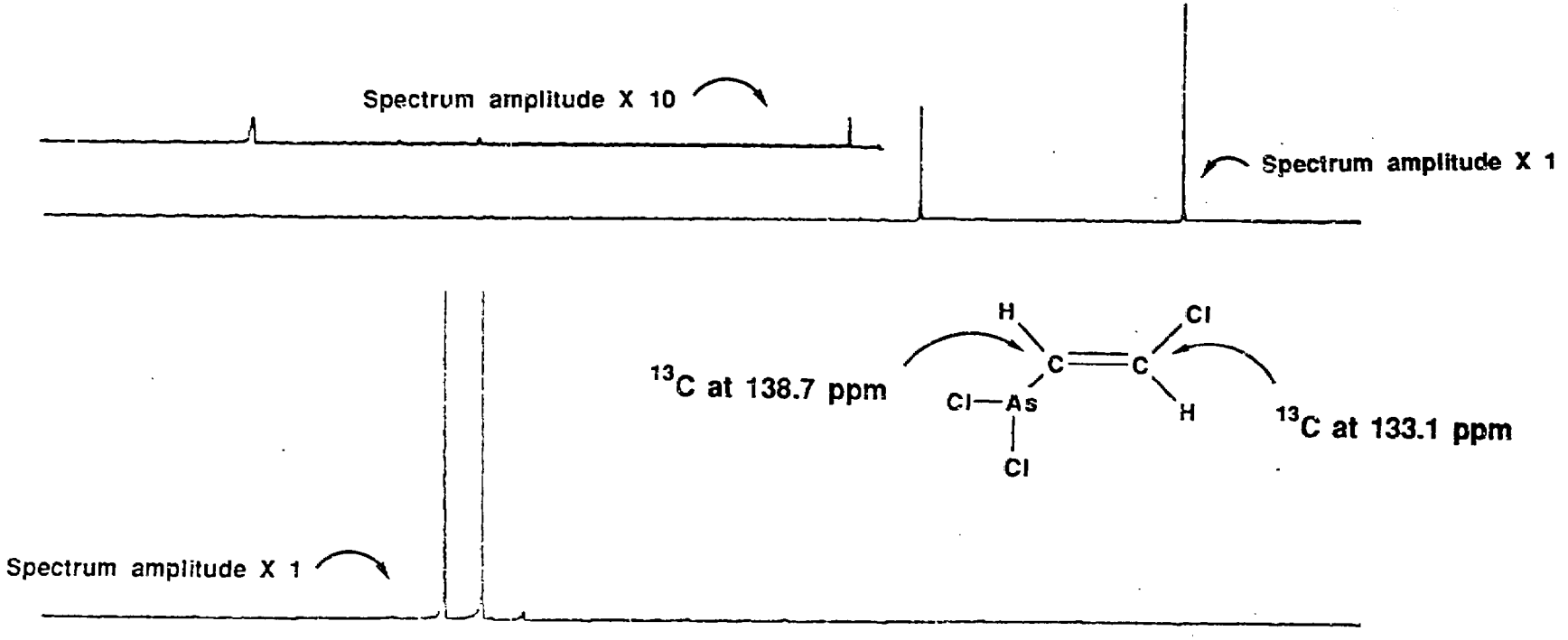
^{13}C at 138.7 ppm

^{13}C at 133.1 ppm

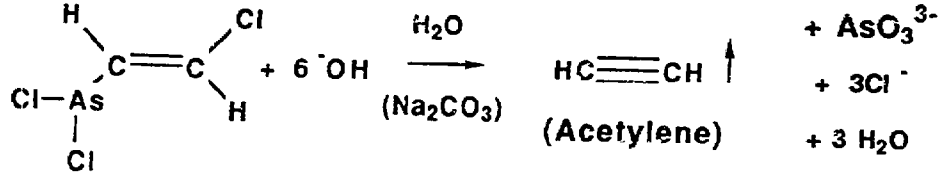
Hydrolysis Stoichiometry:



8.1.18 Lewisite Hydrolysis in 10% Na_2CO_3



Hydrolysis Stoichiometry:



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APPENDIX

8.2 Toxicological Data

8.2.1 Environmental Protection Agency
40 CFR Parts 160 and 792
FIFRA AND TSCA
GOOD LABORATORY PRACTICE STANDARDS

8.2.2 SUMMARY TOXICITY DATA ON
DECONTAMINATED CHEMICAL AGENTS

8.2.3 TYPE PROTOCOL 210880360000

8.2.4 CODE OF FEDERAL REGULATIONS
DEPARTMENT OF TRANSPORTATION
GUIDELINES FOR CLASSES OF POISONOUS MATERIALS

8.2.1 Environmental Protection Agency
40 CFR Parts 160 and 792
FIFRA AND TSCA
GOOD LABORATORY PRACTICE STANDARDS

Monday
December 28, 1987

Part III

Environmental Protection Agency

40 CFR Parts 160 and 792

Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and Toxic Substances Control Act (TSCA); Good Laboratory Practice Standards; Proposed Rules

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 160

[OPP-300165; FRL 3245-5]

Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards

AGENCY: Environmental Protection Agency (EPA).

ACTION: Proposed rule.

SUMMARY: EPA is proposing to expand the scope of the FIFRA Good Laboratory Practice (GLP) Standards by requiring GLP compliance for testing conducted in the field and for such disciplines of testing as ecological effects, chemical fate, residue chemistry, and, as required by 40 CFR 158.160, product performance (efficacy testing). EPA is proposing this amendment in order to ensure the quality and integrity of all data submitted to the Agency in conjunction with pesticide product registration, or other marketing and research permits. EPA is also proposing to amend the FIFRA GLPs to incorporate many of the changes made by the Food and Drug Administration (FDA) to its GLP regulations.

DATE: Submit written comments on or before March 28, 1988.

ADDRESS: Submit written comments, identified by the document control number (OPP-300165), by mail to: Information Services Section, Program Management and Support Division (TS-757C), Office of Pesticide Programs, Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person, deliver comments to: Rm. 236, CM #2, 1921 Jefferson Davis Highway, Arlington, VA.

Information submitted in any comment concerning this proposed rule may be claimed confidential by marking any part or all of that information as "Confidential Business Information" (CBI). Information so marked will not be disclosed except in accordance with procedures set forth in 40 CFR Part 2. A copy of the comment that does not contain CBI must be submitted for inclusion in the public record. Information not marked confidential may be disclosed publicly by EPA without prior notice to the submitter. All written comments will be available for public inspection in Rm. 236 at the address given above, from 8 a.m. to 4 p.m., Monday through Friday, excluding legal holidays.

FOR FURTHER INFORMATION CONTACT: Daniel A. Helfgott, Office of Compliance Monitoring (EN-342), Rm. E 707B, 401 M St. SW., Washington, DC 20460, Telephone: (202) 382-7825.

SUPPLEMENTARY INFORMATION: Following is an index to the remainder of this preamble:

I. Introduction

A. Legal Authority

These standards are promulgated under the authority of sections 3, 5, 6, 8, 18, 24(c), and 25(a) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), 7 U.S.C. 136 et seq., sections 408, 409, and 701 of the Federal Food, Drug, and Cosmetic Act (FFDCA), 21 U.S.C. 301 et seq., and Reorganization Plan No. 3 of 1970.

B. Background

EPA originally published enforceable FIFRA Good Laboratory Practice Standards in the **Federal Register** of November 29, 1983 (48 FR 53446), which were codified as 40 CFR Part 160. At the same time, EPA published GLP standards applicable to testing required under the Toxic Substances Control Act (TSCA, 48 FR 53922, 40 CFR Part 792). These regulations were promulgated in response to investigations by EPA and FDA during the mid-1970s which revealed that some studies submitted to the Agencies had not been conducted in accordance with acceptable laboratory practices. Some studies had been conducted so poorly that the resulting data could not be relied upon in EPA's regulatory decision-making process. For instance, some studies had been submitted which did not adhere to specified protocols, were conducted by underqualified personnel and supervisors, or were not adequately monitored by study sponsors. In some cases results were selectively reported, underreported, or fraudulently reported. In addition, it was discovered that some testing facilities displayed poor animal care procedures and inadequate record-keeping techniques. The FIFRA GLP standards specify minimum practices and procedures which must be followed in order to ensure the quality and integrity of data submitted to EPA in support of a research or marketing permit for a pesticide product.

When EPA published its final FIFRA and TSCA GLP standards in the **Federal Register** of November 29, 1983, the Agency sought to harmonize the requirements and language with those regulations promulgated by the FDA in the **Federal Register** of December 22, 1978 (43 FR 60013), and codified as 21 CFR Part 58. Differences between the two Agencies' current GLP regulations exist only to the extent necessary to reflect the Agencies' different statutory TSCA, FIFRA, and the Federal Food, Drug, and Cosmetic Act (FFDCA) responsibilities. Similar to the FDA GLP regulations, the FIFRA and TSCA GLPs delineate standards for studies designed to determine the health effects of a test substance; however, the TSCA GLPs also contain provisions related to environmental testing (i.e., ecological effects and chemical fate).

Compliance with EPA's GLP regulations has been monitored through a program of laboratory inspections and study audits coordinated between EPA and FDA. Under an Interagency Agreement originating in 1978, FDA carries out inspections at laboratories which conduct health effects testing. EPA primarily performs laboratory inspections and data audits for environmental studies.

After a thorough review of its GLP regulations and compliance program, FDA concluded that some of the provisions of the GLPs needed to be clarified, amended, or deleted in order to reduce the regulatory burden on testing facilities. Accordingly, FDA proposed revisions to its GLPs in the **Federal Register** of October 24, 1984 (49 FR 43530), which were intended to simplify the regulation without compromising study integrity. FDA's proposed revision has recently been published as a final rule in the **Federal Register** of September 4, 1987 (52 FR 33768).

EPA agrees with FDA that many provisions of the GLP regulations can be streamlined without compromising the goals of the GLPs. Therefore, EPA is proposing to amend the FIFRA GLP standards to incorporate many of the changes recently made by FDA to its GLP regulations. In addition, EPA is proposing to expand the scope of the FIFRA GLPs to include the environmental testing provisions currently found in the TSCA GLPs. EPA's proposed revision to the GLPs also extends the scope of the regulation to include product performance data (efficacy testing) as required by 40 CFR 158.160. In sum, the proposed FIFRA GLPs will allow the Agency to ensure the quality and integrity of all data

submitted in support of pesticide product research or marketing permits. In another notice in this **Federal Register**, EPA is proposing similar changes to the TSCA GLP standards.

C. Consistency With FDA GLP Regulations

It is EPA's policy to minimize the regulatory burden on the public which might arise from conflicting requirements which could be promulgated under different regulatory authorities. In keeping with this policy, the final FIFRA 1983 GLP standards, 40 CFR Part 160, followed the format and, with few exceptions, the wording of FDA's final GLP regulations, 21 CFR Part 58. Differences between the EPA and FDA GLP regulations were based upon varying needs and responsibilities under each Agency's regulatory statutes. This proposed revision to the FIFRA GLPs follows this same policy by conforming to many of the changes FDA made to its GLP regulations, published in the **Federal Register** of September 4, 1987 (52 FR 33768). EPA has varied from FDA's revised GLP regulations only when necessary due to EPA's statutory responsibilities. The most significant differences between the EPA proposal and the FDA revised GLP regulations are the scope of the testing and test systems affected.

More specifically, EPA is proposing to require compliance with the FIFRA GLPs for all studies submitted to the Agency which are intended to support pesticide product research or marketing permits. Under the current FIFRA Good Laboratory Practice regulations, and consistent with the FDA GLP regulations, this Agency only requires GLP compliance for health effects testing. However, unlike FDA, testing required by EPA in support of research or marketing permits may include ecological effects, environmental and chemical fate, and efficacy (as stipulated by 40 CFR 158.160 *Product performance data requirements*), as well as health effects testing. Therefore, in an effort to attain consistency in the quality and the integrity of all data submitted to the Agency, EPA has determined that it is necessary to expand the scope of the FIFRA GLPs to require that all types of testing which are used to obtain data in support of research or marketing permits be conducted in accordance with the proposed GLP standards.

EPA's proposed FIFRA GLP standards also vary from FDA's in their coverage of testing conducted in the field. To ensure the quality and integrity of all data submitted in support of research or marketing permits, EPA believes that GLP standards must apply whenever data collection occurs. Because many of the test data required by EPA are developed in the field, or more accurately in outdoor laboratories (i.e., ground-water studies, air monitoring studies, degradation in soil, etc.), EPA is proposing to include field testing within the scope of these regulations.

This Agency's proposed FIFRA GLPs also differ from FDA's in the scope of the requirements provided for test system care facilities, test system supply facilities, and test system care. Because testing required by FDA is focused on health testing, in which animals are the central test system, it is appropriate for FDA's GLP regulations to focus on requirements for appropriate animal care facilities (21 CFR 58.43), adequate animal supply facilities (21 CFR 58.45), and proper animal care (21 CFR 58.90). However, the broad range of testing required by EPA may involve plants, soils, and microorganisms, as well as animals, for the primary test systems. In order to ensure the quality and integrity of all data submitted to this Agency, it is proposed that § 160.43 *Animal care facilities*, § 160.45 *Animal supply facilities*, and § 160.90 *Animal care* be expanded to cover facilities, handling, and care of all test systems. Accordingly, EPA is proposing that these sections be retitled as follows: § 160.43 *Test system care facilities*, § 160.45 *Test system supply facilities*, and § 160.90 *Animal and other test system care*. Further, in most instances, EPA is proposing to replace the term "animal," which is currently used in the FIFRA GLP regulations, with the broader term "test system." Specifically, this change is proposed in §§ 160.43, 160.45, 160.81, 160.90 and 160.120. These proposed changes are further discussed in Unit I.D. of this preamble.

The remaining differences between the EPA and FDA GLP regulations are described in the preamble to this proposed rule and the preamble to the FIFRA Good Laboratory Practice Standards, published in the **Federal Register** of November 29, 1983 (48 FR 53946). EPA has coordinated this proposal with FDA and has considered comments received on the proposal to amend the FDA GLP regulations (49 FR 43530; October 29, 1984).

D. Proposed Changes to the GLP FIFRA Regulations

1. *Section 160.3 Definitions.* a. EPA proposes to define the term "carrier" to mean any material, such as feed, water, soil, nutrient material, etc., with which the test substance is combined for administration to test organisms. The term "carrier" is currently used in § 160.113 and is not defined. EPA proposes to define this term to clarify it.

b. EPA proposes to conform with the September 4, 1987, FDA GLP regulations by amending the definition of "control substance" to exclude feed and water. EPA agrees with FDA's statement regarding this change (52 FR 33769; September 4, 1987) that "the term control [substance] should be reserved for the discrete substances/articles, and vehicles other than water administered to groups of the test system to provide a basis of comparison with the test [substance]."

FDA contends that, under the current definition of "control substance," because the control group of a test system provides the basis for comparison with a test substance, any substance administered to the control group would be considered a control substance. This would mean that feed and water given to the control group of a study are considered a control substance. For instance, in studies in which the test substance or mixture is administered to the test system orally, through feed or drinking water, gavage, or injection, the feed or water is considered a control substance. As a control substance, the feed or water is subject to § 160.105(a) for substance characterization, § 160.105(b) for testing for stability and solubility, § 160.105(c) for requirements for appropriate storage, § 160.105(d) for retention of reserve samples, and § 160.107 for documentation of receipt and distribution of each batch. EPA agrees with FDA that placing these requirements on the use of feed and water as a control substance in control groups unnecessarily burdens the regulated community and is not essential for ensuring the quality and integrity of the data generated by a study.

However, under 40 CFR Part 160, feed and water used as a carrier for the test and control substances or mixtures are still covered by the applicable sections for the testing and storage of test, control, and reference substances and mixtures. For example, § 160.31(e) requires testing facility management to ensure materials are available as scheduled; § 160.45 requires that test system supply facilities shall be provided to ensure proper feed storage; § 160.81(b)(2) requires Standard Operating Procedures (SOP) for test system care, including nutrition; § 160.90(g) requires periodic analysis of feed and water to ensure that contaminants which would interfere with the study are not present; § 160.120(a)(9) requires the protocol to

describe and/or identify the diet used in the study, including the level of contaminants expected in the dietary materials.

c. EPA also proposes to modify the definition of "control substance" by adding the phrase "for no effect levels." This addition to the definition is being proposed merely to clarify the difference between the term "reference substance" and "control substance." While a control substance is used to determine a baseline comparison to no effect levels, a reference substance is used to determine a baseline comparison to an established effect level.

d. EPA proposes to add and define the terms "experimental start date" and "experimental termination date." "Experimental start date" is proposed to mean the first date the test substance is applied to the test system. Under this definition, as of the experimental start date: (1) Under proposed § 160.105(b), the stability and, if important to the conduct of the experiment, the solubility of the test, control, and reference substances would have been determined, (2) under proposed § 160.113(a)(2), the stability and, if important to the conduct of the experiment, the solubility of the test, control, and reference substance in the mixture would have been determined and; (3) under proposed § 160.120(a)(4), the proposed experimental start date would appear in the protocol.

EPA proposes that "experimental termination date" be defined as the last date on which data are collected directly from the study. Under § 160.120(a)(4) as proposed, EPA would require the proposed experimental termination date to appear in the protocol. EPA considers histopathology after scheduled terminal animal sacrifice to be carried out before the experimental termination date.

Experimental start and termination dates would be expressed as the actual calendar dates, not just time-line increments. Therefore, when determining the proposed experimental start and termination dates, as would be required by proposed § 160.120(a)(4), the submitter should consider any lag time relating to protocol approval and laboratory contracting.

e. EPA proposes to add and define the term "reference substance" to mean any chemical substance or mixture or material other than a test substance that is administered to or used in analyzing the test system in the course of a study for purposes of establishing a basis for comparison with the test substance for known effect levels. EPA proposes the use of the term "reference substance" in the revised FIFRA GLP regulations because of its common usage in environmental testing and, therefore, its proposed use in these regulations.

In this proposed GLP regulation, all the requirements provided for test and control substances would also apply to "reference substances." Accordingly, the term "reference substance" has been added wherever the term "test and control substance" appears in these standards. Specifically, the term "reference substance" is added to proposed § 160.29 (d) through (f); § 160.43(b); § 160.47(a) (1) through (3) and (b); § 160.81(b)(3); the Subpart F heading; § 160.90(c); § 160.105 (a) through (e); § 160.107; § 160.113 (a) and (b); § 160.120(a) (2), (9), and (11); § 160.185(a) (4) and (5); and § 160.195(c).

f. EPA proposes to broaden the definition of the term "study" to be consistent with EPA's proposal to amend these regulations to require GLP compliance for all testing required to be submitted to the Agency in conjunction with a pesticide product's research or marketing permit.

EPA is proposing to delete the phrase "in vivo or in vitro" from the definition of "study." The Agency still intends the requirements of these regulations to apply to "in vivo and in vitro" experiments. However, since the Agency intends these regulations to apply to all studies required to be submitted under FIFRA, including those conducted in the field, EPA feels that including the phrase "in vivo or in vitro" in the definition of "study" is too limiting.

Further, EPA is proposing to delete the term "prospectively" from the definition of "study." In this way, epidemiological studies, which could be "retrospective," will be required to be presented to the Agency in accordance with the GLP standards. EPA recognizes that data used in an epidemiological study may not have been generated in conformance with the FIFRA GLP standards, however, it is EPA's contention that the epidemiological study itself can be conducted and submitted to the Agency in accordance with the GLPs.

EPA is also proposing to delete from the current definition of "study" the following sentence: "The term does not include studies utilizing human subjects or clinical studies or field trials in animals." Again, this change is consistent with EPA's intention to require compliance with GLPs for all studies submitted to the Agency in support of a research or marketing permit for pesticide products, including biomonitoring or efficacy studies. FIFRA does not prohibit pesticide testing on humans (as long as the informed-consent conditions specified in FIFRA section 12(a)(2)(P) are met, and provided the records required by 40 CFR 169.2(j) are maintained). EPA feels that testing that is performed on humans must be conducted in accordance with the GLPs, if that data is submitted to the Agency in support of a marketing or research permit.

It is also proposed that studies designed to determine the physical or chemical characteristics of a test substance be included within the scope of these regulations. Therefore, EPA is proposing that the phrase "or to determine physical or chemical characteristics of a test substance" be deleted from the definition of the term "study." This proposed change is consistent with the definition of the term "study" as it now appears, and as it is proposed to appear, in the TSCA Good Laboratory Practice Standards at 40 CFR Part 792. However, as specified in proposed § 160.135, exclusions from certain GLP requirements are provided for studies related to determining the physical or chemical characterization of a test, control, or reference substance (e.g., studies designed to determine color, odor, physical state, melting point, pH measurement, etc.).

g. EPA proposes to incorporate the FDA definitions for "study completion date" and "study initiation date" in § 160.3. "Study completion date" is proposed to mean the date the final report is signed by the study director. EPA advises that the phrase "close of the study" as used in § 160.33(f), and the phrase "study is completed" as used in § 160.185(b)(3) both refer to the "study completion date." Consistent with this definition, as of that date: (1) Under § 160.33(f), the study director must ensure that all raw data, documentation, protocols, specimens, and final reports are transferred to the archives; (2) after this date under § 160.185(c), corrections or additions to the final report must be in the form of an amendment by the study director under the procedures specified in that section; and (3) in the applicable situations described in § 160.195(b)(3), records must be maintained for a period of at least 2 years following the study completion date.

EPA proposes to define "study initiation date" as the date the protocol is signed by the study director. EPA advises that the phrase "study is initiated" as used in § 160.31(a), and the phrase "study was initiated" as used in § 160.35(b)(1) would refer to the "study initiation date." Therefore, as of the study initiation date: (1) Under § 160.31(a), the testing facility management would designate a study

director; (2) under § 160.35(b)(1), the study would be entered on the master schedule sheet by the quality assurance unit; and (3) under § 160.120(b), after this date all changes or revisions in the protocol would be documented, signed by the study director, and dated. EPA also expects that as of the study initiation date, under § 160.31(e), the testing facility management would have ensured that personnel, resources, facilities, equipment, material, and methodologies are available as scheduled.

h. EPA proposes to replace the term "test substance or mixture" with the term "test substance." This is an editorial change which makes usage consistent in the GLP standards. The term "test substance" is proposed to be defined to include mixtures.

i. EPA proposes to expand the definition of "test system" to include chemical or physical matrices (e.g., soil or water). This proposal is consistent with the Agency's intent to expand these regulations to include environmental effects testing.

j. EPA proposes to define the term "vehicle" to mean any agent which facilitates the mixture, dispersion, or solubilization of a test substance with a carrier.

2. *Section 160.31 Testing facility management.* In conformance with the revised FDA GLP regulations, in § 160.31(b), EPA proposes to delete the requirement that the replacement of a study director must be documented as "raw data." EPA agrees with FDA that this requirement is redundant with other provisions of the GLPs. For instance, § 160.35(b)(1) states that the master schedule sheet must contain the name of the study director. As FDA notes (52 FR 33770), any replacement of the study director would be reflected on the master schedule sheet, which is already considered "raw data." Further, § 160.120(b) states that all changes in an approved protocol must be documented and signed by the study director. Replacement of the study director is considered to be a change in the approved protocol.

3. *Section 160.35 Quality assurance unit (QAU).* a. In § 160.35(a), EPA proposes to conform with the revised FDA GLP regulations by substituting the term "which" for the current phrase "composed of one or more individuals who." This change clarifies that EPA does not require the QAU to be a fixed, permanently staffed unit whose only functions are to monitor the quality of a study. The Agency is only concerned that there be a distinct separation of duties between those personnel involved with the conduct or direction of a study and those personnel performing quality assurance on the same study. Therefore, EPA does intend proposed § 160.35(a) to prohibit personnel from performing quality assurance activities on their own study.

b. In § 160.35(b)(1) EPA proposes to delete the requirement that the name of the study sponsor appear on the master schedule sheet. Instead, it is proposed that under § 160.35(b)(1) the sponsor's identity appear on the master schedule sheet. This change is being proposed to be consistent with the FDA's recent revision and to provide the regulated community the option of using an identity code on the master schedule in lieu of the sponsor's name.

EPA agrees with FDA's contention that requiring the sponsor to be identified specifically by name on the master schedule is not essential to fulfill the requirements of the GLPs or the goal of ensuring the quality and integrity of the data generated from the studies. However, while the name of the study sponsor would not be required to appear on the master schedule sheet, this information must be made available to the Agency upon request.

c. As in the revised FDA GLP regulations, EPA is also proposing to delete the requirement in § 160.35(b)(1) that the master schedule sheet contain the status of the final report. EPA agrees with FDA that this requirement is redundant in view of the other information required by § 160.35(b)(1) such as the date the experiment began and the current status of each study.

d. In conformance with the revised FDA GLP regulations, EPA proposes to modify the requirements of § 160.35(b)(3) to provide for inspections of a study on a schedule adequate to ensure the integrity of the study. This section currently specifies that the quality assurance unit must inspect each phase of a study periodically. This section also currently specifies that for studies lasting more than 6 months, quality assurance inspections shall be conducted every 3 months, and for studies lasting less than 6 months, quality assurance inspections shall be conducted at intervals adequate to ensure the integrity of the study.

The proposed changes to this section will allow the QAU the necessary latitude to adjust its monitoring activities to meet the individual problems of each study. EPA agrees with FDA's contention that an inspection of each phase of the study is not necessary to ensure that a study is being conducted properly. However, EPA also agrees with FDA that each study, no matter how short, must be inspected at least once while in progress. EPA expects that by allowing the QAU flexibility in designing a reasonable inspection schedule, the goal of ensuring the quality of the study can be best achieved.

e. Consistent with the revised FDA GLPs, EPA is proposing to delete § 160.35(e) in its entirety. Section 160.35(e) currently requires that all quality assurance records be kept in one location at the testing facility. As FDA pointed out in its October 29, 1984, proposed GLP revision, since § 160.190(b) already requires the use of archives for the orderly storage and expedient retrieval of all reports and records, the requirements of § 160.35(e) are not necessary. However, EPA maintains that all reports and records, including those of the QAU, must be easily accessible and made available to EPA and FDA inspectors when requested.

4. *Section 160.41 General.* FDA has deleted from its GLPs the requirement that the location of each testing facility be suitable to facilitate the proper conduct of studies. However, EPA is proposing that § 160.41 require that testing facilities which are not located within an indoor controlled environment be suitably located to facilitate the proper conduct of studies.

The studies FDA requires are generally conducted within the confines of a traditional indoor laboratory. Because the conditions specified within a protocol can be artificially manipulated within the traditional indoor laboratory, the location of these laboratories is generally not a factor in determining the quality of a study. Therefore, it is not necessary to ensure that a traditional indoor testing facility is suitably located to facilitate the proper conduct of the study.

However, the studies EPA requires are not necessarily conducted within the confines of the traditional indoor scientific laboratory (i.e., field studies, groundwater studies, ecological toxicity studies, etc.). EPA considers any site where testing is undertaken to generate data required by the Agency to be a testing facility. The conditions required by the protocol are not necessarily conducive to artificial manipulation in the field, or other outdoor testing facilities. Therefore, ensuring the suitability of the location of these types of testing facilities is both a valid and necessary part of EPA's GLP Standards.

5. *Section 160.43 Test system care facilities.* a. EPA is proposing to revise the title of § 160.43 from "Animal care facilities" to "Test system care facilities". The proposed heading for § 160.43 more adequately reflects the

Agency's intent to specify facility requirements for the care of chemical or physical matrices (e.g., soil or water), plants, and microorganisms, as well as animals. Accordingly, the Agency is proposing to further modify § 160.43 by incorporating the term "test system" when facility requirements should extend beyond "animal" care.

Consistent with the Agency's intent stated above, paragraphs (a)(1), (a)(2), (d), (e), (f), (g), and (h) in proposed § 160.43 have been added or modified in order to ensure proper care facilities are provided for the additional test systems covered by the expanded section.

b. EPA proposes to modify § 160.43(a) to allow testing facilities to provide for isolation areas rather than quarantine areas. This change is consistent with the proposal to modify § 160.90(b) to allow "isolation" of newly received animals rather than require "quarantine" [See Unit I.D. of this preamble for a discussion of proposed § 160.90(b)].

c. In § 160.43(c), EPA proposes to delete the requirement that separate areas be provided in all cases for the diagnosis, treatment, and control of test system diseases. Instead, it is proposed that such separate areas be provided "as appropriate." This proposal is consistent with the September 4, 1987, revised FDA GLP regulations.

EPA has proposed this modification in order to allow laboratories the option of disposing of diseased animals and other test systems without also bearing the expense of maintaining separate areas in testing facilities for diagnosis, treatment, and control of disease. Additionally, EPA recognizes that the diagnosis and treatment requirements of § 160.43(c) may not be appropriate when dealing with such test systems as soil, plants, or microorganisms. However, if the decision is made not to dispose of the test system, then test system care facilities, as specified in proposed § 160.43(c), must be provided.

d. EPA proposes to conform to the revised FDA GLPs by deleting § 160.43(e) in its entirety. Currently, § 160.43(e) requires test system facilities to be designed, constructed, and located so as to minimize disturbances which may interfere with the study. EPA agrees with FDA that this provision is already adequately covered in § 160.41, which requires that facilities be of suitable size, construction, and, for outdoor testing facilities, location to facilitate the proper conduct of the study.

6. *Section 160.45 Test system supply facilities.* a. EPA proposes to expand the scope of § 160.45 to require that supply facilities necessary for environmental testing be provided when appropriate.

b. Consistent with the proposed expanded scope of this section, EPA is also proposing to retitle § 160.45, from "Animal supply facilities" to "Test system supply facilities."

c. EPA also proposes to modify § 160.45 to state "Perishable supplies shall be preserved by appropriate means." This change is being proposed to conform with the revised FDA GLPs and recognizes that there are a variety of acceptable storage and preservation procedures available besides refrigeration. Depending on the stability characteristics of the perishable material, acceptable storage and preservation methods may include desiccation, room temperature-low humidity, and constant temperature-low humidity.

d. EPA also proposes to delete the phrase "or feed" from the last sentence of § 160.45. Both EPA and FDA consider "feed" to be a "supply." Therefore, the use of the word "feed" in § 160.45 is redundant.

7. *Section 160.49 Laboratory operation areas.* a. EPA proposes to conform with FDA's revised GLP regulations by deleting paragraph (b) from § 160.49, adding the phrase "and specialized" after the word "routine" and before the word "procedures," and deleting the qualifying phrase "including specialized areas for performing activities such as aseptic surgery, intensive care, necropsy, histology, radiography, and handling of biohazardous materials."

Paragraphs (a) and (b), as currently worded, describe activities which require that separate laboratory space be provided. As FDA noted in its proposal to modify its corresponding section (49 FR 43532), the list of activities that currently appears in paragraphs (a) and (b) is not all inclusive and is not essential for the clarity of these sections. Further, by adding the phrase "and specialized," the proposed new paragraph will encompass all activities now listed in paragraphs (a) and (b).

b. In § 160.49, EPA proposes to add the phrase "and other space" after the words "laboratory space" and before the word "shall." As discussed in Unit I.C. of this preamble, this change to § 160.49 is being proposed to reflect that testing does not necessarily take place within the confines of a traditional indoor laboratory. Proposed § 160.49 would require that there be enough space provided to perform the procedures required by the protocol Wherever testing takes place (i.e., indoor laboratory or field station).

8. *Section 160.53 Administrative and personnel facilities.* As in the revised FDA GLP regulations, EPA proposes to delete § 160.53 in its entirety. EPA agrees with FDA that the requirements of this section are not necessary for achieving the goals of the FIFRA GLP standards.

9. *Section 160.61 Equipment design.* In § 160.61, EPA proposes to delete the phrase "Automatic, mechanical, or electronic" from the beginning of the first sentence. EPA agrees with FDA that the deletion of these qualifying terms provides for a more general interpretation of the word "equipment."

10. *Section 160.63 Maintenance and calibration of equipment.* a. Consistent with the FDA GLPs, EPA is proposing to amend § 160.63(b) to state that standard operating procedures (SOPs) for remedial action for equipment, in the event of failure or malfunction of equipment, need only be established when "appropriate." This change acknowledges that laboratories may choose to discard rather than repair equipment, and in such cases SOPs which delineate remedial action are not necessary.

b. EPA is also proposing to conform to the revised FDA GLP regulations by deleting from § 160.63(b) the provision that copies of the SOPs shall be made available to laboratory personnel. EPA still believes that laboratory personnel must have access to laboratory SOPs; however, since this requirement is clearly stated in § 160.81(c), EPA considers the inclusion of this requirement in § 160.63(b) to be redundant.

11. *Section 160.81 Standard operating procedures.* a. In § 160.81(b) (1), (2), (6), (7), and (12), EPA is proposing to replace the term "animal" with the term "test system." As discussed previously in this preamble, this modification is consistent with the broad scope of test systems which may be used in testing undertaken in support of a pesticide product research or marketing permit.

b. In § 160.81(b)(5), EPA is proposing to require that SOPs be established for tests wherever the testing is undertaken, including those conducted in the field. Accordingly, it is proposed that § 160.81(b)(5) read "Laboratory or other tests" (see discussion of "field testing" in Unit I.C. of this preamble).

c. In conformance with FDA's revised GLP regulations, EPA is proposing to delete the list of examples for laboratory manuals and SOPs required to be made immediately available under § 160.81(c). EPA still intends that laboratory areas must have immediately available manuals and SOPs for laboratory procedures being performed. This requirement still includes toxicology, histology, clinical chemistry,

hematology, teratology, and necropsy, if applicable. However, this list is not all inclusive and is too broad to serve as a useful guide. For example, this requirement also includes SOPs for the maintenance, repair, and calibration of equipment as described in § 160.63(b).

d. EPA is also proposing to amend the language of § 160.81(c) to clarify that the requirement of this section also applies to field testing facilities. Therefore, it is proposed that § 160.81(c) will read, "Each laboratory *or other study* area shall have immediately available manuals and standard operating procedures relative to the laboratory or field procedures being performed."

12. *Section 160.90 Animal and other test system care.* a. EPA is proposing to retitle § 160.90 from "Animal care" to "Animal and other test system care". As previously stated, testing required by EPA may involve plants, soils, microorganisms, and other test systems, in addition to animals. The proposed title to § 160.90 reflects the broader scope of this Agency's regulatory responsibilities, these regulations, and this section, to provide for the quality and integrity of all data submitted in support of pesticide product research and marketing permits.

Consistent with the Agency's proposal stated above, paragraphs (b), (d), (e)(1), (f), (g), and (j) in proposed § 160.90 have been added or modified in order to ensure the proper care of all test systems used in a study.

b. EPA proposes to modify § 160.90(b) to provide for the evaluation of a test system's health status, or the appropriateness of the test system for the study, according to acceptable "scientific practice." This section, as proposed, will still require that newly received animals must have their health status evaluated according to acceptable veterinary medical practices. However, EPA recognizes that it may not be appropriate to evaluate the health status of certain test systems (e.g., soil or water) or to require that a plant, microorganism, soil, or water be evaluated according to acceptable veterinary medical practice to determine their appropriateness for a study. EPA is only concerned that test systems used in a study are free of any disease or condition which may interfere with the purpose or conduct of the study, and that the proper precautions, as stated in § 160.90(b), are taken to comply with this requirement.

c. Additionally, EPA is proposing to modify § 160.90(b), to require "isolation" rather than "quarantine" of newly received animals. This proposal is consistent with FDA's revision to its GLPs.

As previously stated, the intent of § 160.90(b) is to prevent the entry of unhealthy or inappropriate test systems into the study, as required by § 160.90(c). Currently, § 160.90(b) provides that this intent be achieved through "quarantine." However, the term "quarantine" suggests a rigid set of procedures, including a mandatory holding period, a specific list of diagnostic procedures, and the use of specialized facilities and test system care practices, which may be an unnecessary burden to industry.

EPA agrees with FDA's conclusion, discussed in the preamble to its revised GLPs (52 FR 33775; September 4, 1987), that isolation and evaluation of health status are sufficient precautions against contamination of test systems and, therefore, fulfill the intent of this section. FDA further states that such a revision would provide laboratories the flexibility to develop isolation and health status evaluation procedures best suited for the age, species, class, and type of the test system, as well as the type of study to be performed.

d. EPA proposes to conform to the FDA GLPs by modifying § 160.90(c) to require isolation of diseased test systems only when necessary.

Currently, § 160.90(c) requires that animals which contract a disease or condition shall be isolated in all cases. This requirement would in turn require that separate facilities be available for the isolation of these animals. However, as discussed in the proposal for § 160.43(c), both EPA and FDA believe that laboratories should be given flexibility in their disposition of diseased test systems. As FDA discussed in the proposed revisions to its GLP regulations (49 FR 43533; October 29, 1984), the proposed modification to § 160.90(c) will allow laboratories the option of: (1) Leaving the diseased test system in the experiment provided that the integrity of the study will not be adversely affected by this action; (2) disposing of the test system; or (3) isolating treating, and returning the test system to the study.

13. *Section 160.105 Test, control, and reference substance characterization.* a. In revised 21 CFR 58.105(a), FDA deleted the requirement that test and control substance characteristics shall be determined and documented for each batch "before the initiation of the study." This change has not been adopted by EPA in its proposed revision to § 160.105(a). However, EPA proposes to modify § 160.105(a) to require that test, control, *or reference* substance characterization be determined and documented for each batch before its use in the experiment. EPA feels that this proposed requirement is necessary because it is essential that characteristics of test, control, and reference substances be known prior to their administration or use in an experiment.

EPA's recent experience with antimony trioxide has shown that extensive analytical work was necessary prior to test initiation. Certain assumptions regarding the product's characteristics were used in the protocols for antimony trioxide testing which proved invalid. These invalid assumptions necessitated modifications to the proposed study, resulting in the delay and rescheduling of other subsequent studies. If the analytical work had preceded the toxicology studies, the studies would not have failed and modifications to the studies would not have been necessary. The Agency's conclusion is that it is better to delay study schedules than to initiate improper experimental procedures which will produce invalid results.

b. FDA has modified 21 CFR 58.105(b) to provide for the determination of the stability of the test or control substance either before the initiation of the study or through periodic analysis of each batch according to written standard operating procedures. EPA has chosen not to adopt this approach in proposed § 160.105(b) because the Agency does not agree that stability can adequately be demonstrated by periodic analysis without initial evaluation.

Further, there are many studies required by EPA where solubility of the test, control, or reference substance is of critical importance, such as aquatic toxicity studies. Therefore, EPA is proposing that solubility of the test, control, or reference substance be determined before the experimental start date if knowledge of solubility characteristics is relevant for the proper conduct of the experiment.

It is EPA's contention that both stability and solubility of the test, control, and reference substance need to be determined before the experimental start date in order to ensure proper handling and administration of the test substance to the test system. However, since the determination of the solubility of the test, control, and reference substance is not a requirement in FDA's GLP regulations, EPA is interested in receiving public comment on this issue.

14. *Section 160.113 Mixtures of substances with carriers.* a. FDA has revised 21 CFR 58.113(a)(2) to require determination of the stability of the test and control substance in a mixture, as required by the conditions of the study, either before the initiation of the study

or through periodic analysis of each batch. While EPA does not propose to modify § 160.113(a)(2) to provide the option of determining the stability of the mixture either before study initiation or through periodic analysis (see discussion for § 160.105(b)), EPA will modify this section to require stability testing only to the extent required by the conditions of the experiment. As proposed for § 160.105(b), EPA is also proposing to require that, when appropriate to the conduct of the experiment, solubility of the test, control, or reference substance in the mixture be determined in the same manner (see discussion for § 160.105(b)). Additionally, as proposed for § 160.105 (a) and (b), EPA is proposing to replace the phrase "before the initiation of the study" with the phrase "before the experimental start date" (see discussion for § 160.105(a)).

The phrase "as required by the conditions of the experiment" has been added in order to clarify that determination of stability and, if appropriate, solubility of a test, control, or reference substance in a mixture is only necessary to support the actual time of use in the experiment. Therefore, it is not necessary to provide data which illustrate long-term stability of a mixture when the actual time that the mixture is used is short-term. For example, a test, control, or reference substance in a mixture that will be used the same day it is prepared will only require data sufficient to show stability and, if appropriate, solubility for 1 day.

b. EPA proposes to add § 160.113(c) which states, that if a vehicle is used to facilitate the mixing of a test substance with a carrier, assurance shall be provided that the vehicle does not interfere with the integrity of the test.

15. *Section 160.120 Protocol.* a. In revised 21 CFR 58.120(a), FDA has replaced the qualifying phrase "but shall not necessarily be limited to" with the phrase "as applicable." EPA proposes to adopt FDA's approach with some modifications. It is proposed that the phrase "Where applicable" appear before the information specified in § 160.120(a)(9), and continue to appear before the information required by § 160.120(a)(6). The phrase "but shall not necessarily be limited to" would remain in this section.

In FDA's discussion of this proposal (49 FR 43533; October 29, 1984), concerns were expressed that some of the information required to appear in the protocol is not applicable to all types of testing. Specifically, FDA points to the information required by 21 CFR 58.120(a) (9) and (11). In 21 CFR 58.120, paragraph (a)(9) requires a description of the diet used in a study as well as solvents, emulsifiers, and/or other materials used to solubilize or suspend the test or control substance before mixing with the carrier. FDA points out that this requirement is not applicable to radiation-emitting products. Section 58.120(a)(11) specifies that the protocol shall specify dosage level, and this requirement is not applicable to implantable medical devices.

Clearly, the basis for FDA's change is to accommodate concerns that are specific to the types of testing required by FDA and do not necessarily apply to testing required by EPA. Further, EPA is concerned that placing the phrase "as applicable" in § 160.120(a) suggests that there may be cases where it is not applicable for any of the other information required by § 160.120(a) to appear in the protocol. Therefore, the phrase "as applicable" should only appear before those items which are not necessarily appropriate to appear in the protocol for certain types of testing.

For example, there may be testing required by EPA where it may not be appropriate to require a protocol to contain the information specified in § 160.120(a)(9), such as describing and/or identifying the diet of a human subject involved in exposure testing. Therefore, EPA proposes to add the phrase "Where applicable" before the information specified in proposed § 160.120(a)(9).

b. In revised 21 CFR 58.120(a)(4), FDA has deleted the requirement that the protocol contain "The proposed starting and completion dates." EPA is proposing to retain this requirement in § 160.120(a)(4), but is proposing to modify this paragraph to require, "The proposed experimental start and termination dates."

EPA believes that this information is necessary for the evaluation of a protocol, and the Agency scheduling of additional related studies and audit reviews. Section 160.120(a)(4) is related to the selected study method, laboratory, and specialist availability, and other Agency and industry priorities. Often a group of experiments are carried out in sequence, so that both start and termination dates affect subsequent study expectations and timetables. Projected experimental start and termination dates identify the normal duration for a given experiment type and reflects any special considerations that may be unique to a laboratory, anticipated analytical or methodology work, and available resources, and it may also affect pending regulatory timetables.

Given that there are hundreds of studies that EPA must track, these estimated schedules, combined with those from other studies, allow the Agency to more efficiently schedule audits and regulatory action. Further considerations are the following: (1) The availability of composite schedules for many studies may be necessary to set realistic regulatory action goals; (2) composite study schedules are evaluated to schedule audits while several studies are ongoing or recently completed, and which may all be at a given laboratory or geographic location, thus directly reducing EPA resources necessary for audit and regulatory review functions; and (3) standard business management by objectives requires intermediate calendar goals when scheduling multiple outputs, or a long-term single product. The master on-site laboratory schedule will incorporate these dates to carry out the study.

c. In 21 CFR 58.120(a)(5), FDA has deleted the requirement that the protocol contain a justification for the selection of the test system. EPA has chosen to leave this requirement in proposed § 160.120(a)(5).

Environmental studies, including both ecological effects and chemical fate, are more diverse than health effects testing. Further, details relevant to the test system design are more chemically dependent in the case of environmental effects and chemical fate testing than in the case of health effects testing. Many of the test systems in environmental studies must be modified in accordance with specific chemical characteristics. Therefore, EPA must allow a much broader range of flexibility in the nature of tests and selection of test systems. In order to fully understand the test and its results, EPA needs to have a discussion of the reasons for selection of the test system. In addition, EPA recognizes that industry may be engaged in state-of-the-art environmental testing. Under proposed § 160.120(a)(5), EPA can keep abreast of industry advances in such testing and ensure that their use of test systems is appropriate. EPA is interested in receiving public comment on whether to limit the requirement that the protocol contain a justification of the test system to environmental testing.

d. FDA has deleted from 21 CFR 58.120(a)(10) the requirement that the protocol include the route of administration and the reason for its choice. EPA has chosen to retain this requirement in proposed § 160.120(a)(10).

The chemicals regulated by FDA will usually have a predefined route of exposure. Therefore, it makes sense for FDA to eliminate the requirement to stipulate the route of administration and

the reason for its choice within the protocol. Unlike FDA, EPA is concerned with presence in or exposure to various media (i.e., air, water, soil, sediment, chemicals, etc.) and may not know in advance the routes of exposure for the chemicals it regulates. Most chemicals and products regulated by EPA do not have set routes of exposure and may even have multiple routes of exposure. Therefore, EPA must consider a wide range of possible exposure routes in its regulatory decisions. Further, the route of administration is essential to determine the effectiveness of a test system for the purposes of a specific toxicology study. The route of administration affects the real dosage rates and, therefore, affects whether the impact of the exposure of the test substance is acute or chronic.

Therefore, EPA believes that, for its purposes, it is essential that the protocol contain the route of administration and the reason for its choice. This requirement will therefore remain in the FIFRA GLPs in § 160.120(a)(10).

e. EPA proposes to delete current § 160.120(a)(12) in its entirety. Currently, § 160.120(a)(12) requires that the protocol contain the method by which the degree of absorption of the test and control substance by the test system will be determined. EPA agrees with FDA's conclusion that this requirement is not necessary in the protocol.

f. In proposed § 160.120(a)(14), redesignated from current paragraph (a)(15), EPA proposes to conform with FDA's revised GLP regulations and require that the study director's signature be dated on the protocol.

EPA is proposing in § 160.3 that the study initiation date be defined as the date the protocol is signed by the study director. It is through the proposed requirement of § 160.120(a)(14), that the Agency will be able to identify the official study initiation date.

16. *Section 160.130 Conduct of a study.* a. FDA has modified 21 CFR 58.130(d) to provide that records of gross findings for a specimen from postmortem observations "should" be made available to the pathologist when examining that specimen's histopathology. EPA is proposing to retain the requirement that these records "shall," in all cases, be provided to a pathologist during study of the specimen.

EPA agrees with FDA's conclusion that for most studies it is important for the pathologist to have the records of gross findings available when examining a specimen histopathologically. However, it is FDA's contention that replacing the word "shall" with the word "should" will allow the histopathological evaluation of specimens in a "blind" fashion. EPA also recognizes that it may be appropriate for some studies to provide for "blinding" in histopathological evaluation. However, EPA maintains that, when specified by the protocol, the pathologist can accomplish "blinding," without violating § 160.130 by not looking at the records which have been provided. Therefore, it will remain EPA's requirement that the pathologist must have access to the records of gross findings when examining a specimen histopathologically.

b. In conformance with the revised FDA GLP regulations, in § 160.130(e), EPA proposes to replace the terms "computer" and "computer driven" with the term "automated data collection." EPA agrees with FDA that the terms "computer" or "computer driven" do not adequately reflect the data collection and storage technologies currently used by testing facilities. The Agency believes that the proposed term "automated data collection" provides a more appropriate description of the data collection and storage systems available for industry use.

17. *Section 160.135 Physical and chemical characterization studies.* EPA proposes to add § 160.135 in order to specify the provisions of the proposed FIFRA GLP standards which will not apply to studies designed to determine the physical and chemical characteristics of a test, control, or reference substance. Most studies designed to determine the physical or chemical characteristics of a test, control, or reference substance rarely involve any modifications to the protocol or experimental design and are usually conducted in an assembly line fashion. Therefore, proposed § 160.135(a) relaxes the requirements of the GLP standards without compromising the quality or integrity of data generated from these studies.

However, in § 160.135(b), EPA is also proposing that the exemptions listed in proposed § 160.135(a) will not apply to studies designed to determine stability, solubility, octanol water partition coefficient, volatility, and persistence of a test, control, or reference substance. These types of physical and chemical characterization studies are more complex in design, execution, and interpretation, and EPA does not believe that it can be assured of the quality and integrity of data generated from these studies without complete GLP compliance.

18. *Section 160.185 Reporting of study results.* a. In § 160.185(a)(5), EPA is proposing to require that the final report include information relating to the solubility, in addition to stability, of the test, control or reference substance if solubility information was important to the conduct of the experiment. This change is consistent with the proposed modification to §§ 160.105(b) and 160.113(a)(2) (see discussion of proposed §§ 160.105(b) and 160.113(a)(2)).

19. *Section 160.190 Storage and retrieval of records and data.* a. In § 160.190(a), EPA proposes to conform to the revised FDA GLP regulations by modifying this section to state that specimens obtained from mutagenicity tests and specimens of blood, urine, feces, and biological fluids generated as a result of a study need *not* be retained. EPA is also proposing that § 160.190(a) state that specimens of soil, water, and plants obtained from environmental testing need *not* be retained. EPA agrees with FDA's conclusion that retention of these specimens beyond initial evaluation is burdensome and does not have a significant impact on the quality of a study.

b. As in the revised FDA GLPs, EPA proposes to revise § 160.190(e) by deleting the requirement that study materials which are retained in archives must be indexed specifically by test substance, date of study, test system, and nature of study. EPA agrees with FDA that the intent of this section is to require indexing of materials in such a way as to permit expedient retrieval from archives. EPA does not believe it is necessary to stipulate the specific indexing terms which must be used.

20. *Section 160.195 Retention of records.* a. In § 160.195, EPA proposes to delete the examples provided in the first sentence of paragraph (c). EPA has proposed this change in conformity with FDA's recent revision because EPA agrees with FDA that these examples do not clarify which materials must be retained from a study, and therefore, are not necessary in this section.

b. EPA is also proposing to modify § 160.195(c) to state that specimens obtained from mutagenicity tests, specimens of soil, water, and plants, and wet specimens of blood, urine, feces, biological fluids, do not need to be retained beyond quality assurance review. This change has been adopted in order to be consistent with the change discussed in proposed § 160.190(a).

c. In new § 160.195(i), EPA proposes to allow records and other "raw data" required by these regulations to be retained either as original records or as true copies, such as photocopies, microfiche, or other accurate reproductions of the original records. This provision would be incorporated in the FIFRA GLPs, in § 160.195(i), in order

to be consistent with the recent changes to FDA's Good Laboratory Practice Regulations.

II. Economic Analysis

In order to satisfy requirements for analysis as specified by Executive Order 12291 and the Regulatory Flexibility Act, the Agency developed a document entitled "Regulatory Impact Analysis of the FIFRA Good Laboratory Practices Regulations". This document, which is available for public inspection, estimates the costs of compliance with the proposed revisions to the FIFRA Good Laboratory Practices Regulations. Compliance costs were estimated using data from a survey of laboratories potentially affected by the revised GLP regulation and from data on pesticides testing demand and costs taken from a 1980 study of the pesticides testing industry.

It was found that the GLP revisions will not increase the costs of health effects testing and that non-health effects testing costs will increase by about 20 percent. It is estimated that the adoption of the proposed GLP revisions would increase annual pesticide testing costs by between $6.3 and $9.9 million in 1986 dollars.

III. Statutory Requirements

As required by FIFRA section 25, copies of this proposed rule were provided to the Scientific Advisory Panel, the Secretary of Agriculture, the Senate Committee on Agriculture, Nutrition, and Forestry, and the House Committee on Agriculture. No comments were received from either Congressional Committee and the FIFRA Scientific Advisory Panel waived its review of this proposal. The following are the comments of the Secretary of the Department of Agriculture and the response of EPA:

The Secretary of the Department of Agriculture requested that the definition of "study" be modified to more clearly reflect EPA's intent that GLP compliance for efficacy testing be limited to product performance as required by 40 CFR 158.160. We have modified the definition of "study" accordingly.

The Secretary asked if the regulation requires that only studies conducted in accordance with the GLPs are acceptable for Agency review and, are there any conditions under which a study can be accepted which did not fully comply with the GLPs?

Studies may be submitted to EPA which do not completely conform to the GLPs as long as the Statement of Compliance, required by § 160.12(b) of the GLP regulations, describes in detail all differences between the practices used in the study and those required by the GLPs. EPA will review these studies. However, EPA will decide on a case-by-case basis whether studies which deviate from the GLPs are acceptable to support the pesticide product registration, or other marketing and research permit.

The Secretary of Agriculture asked if studies which reflect negatively on a chemical use, or studies which report toxic or carcinogenic effects will automaticaliy be ignored by EPA if they have not been conducted under verifiable GLP conditions.

EPA will *not* ignore scientific data which does not comply with the FIFRA GLP standards, and may choose to rely on such data for purposes of showing adverse effects. However, as stated by § 160.17(a) of the FIFRA GLPs, EPA may determine that data which does not comply with the GLPs is not reliable to support an application for a research or marketing permit. Further, § 160.15(b) of the GLPs states that "The determination that a study will not be considered in support of an application for a research or marketing permit does not, however, relieve the applicant for such a permit of any obligation under any applicable statute or regulation to submit the results of the study to EPA." Adverse effects data, which is required to be submitted to the Agency under FIFRA section 6(a)(2), must be submitted to the Agency regardless of whether it complies with the GLPs or not. The Agency does not now, and will not in the future, require FIFRA section 6(a)(2) data to be generated and submitted to the Agency in accordance with the GLPs. EPA will not ignore any FIFRA section 6(a)(2) data. However, additional testing required by the Agency as a result of the FIFRA section 6(a)(2) finding must be conducted in accordance with the GLPs.

The Department of Agriculture commented that if they are required to conduct the analyses described in §§ 160.105 and 160.113, it would greatly limit their resources and capability to conduct studies under the minor use pesticide program. They state that they are working with labeled pesticides which already have tolerances established in food crops, and that are being utilized under simulated commercial conditions. Therefore, they believe that the information gained from these analyses would not be of any real significance to the results of the studies for efficacy, phytotoxicity, and residue.

EPA continues to believe that adequate test, control, and reference substance characterization, and knowledge of their behavior in the mixture, is essential to assure the quality and integrity of the test. EPA agrees that the analyses of a test, control, or reference substance mixed with a carrier, as required by § 160.113, may be costly. However, some cost savings can be realized by obtaining the documentation of the identity, strength, purity, and composition for each batch of the test, control, and reference substance, as required by § 160.105, from the manufacturer of these chemicals (this is particularly pertinent when the chemical is specifically synthesized for the test). These analyses do not have to be repeated by the testing facility.

Finally, please note, the analyses required by §§ 160.105 and 160.113 are only required for efficacy testing of the types of products specified by 40 CFR 158.160 (e.g., for pesticide products that claim to control microorganisms that pose a threat to human health and pesticides that claim to control vertebrates that may transmit diseases to humans). Therefore, in most cases efficacy testing that is conducted under the Department of Agriculture's minor use pesticide program is not required to comply with the requirements of the GLPs, including the analyses required by §§ 160.105 and 160.113.

Finally, the Secretary of the Department of Agriculture asked if there is a grandfather provision for studies conducted prior to the implementation of the regulations.

EPA does not intend to require compliance with the revised GLP standards for studies begun significantly before the effective date of the final version of these proposed regulations.

IV. Other Regulatory Requirements

A. Executive Order 12291

Under Executive Order 12291, EPA is required to judge whether a rule is a "major" one and is therefore subject to the requirement of a Regulatory Impact Analysis. The proposed amendments of the FIFRA Good Laboratory Practice Standards would not be a major rule because they do not meet any of the criteria set forth and defined in section 1(b) of the Order.

B. Regulatory Flexibility Act

This rule has been reviewed under the Regulatory Flexibility Act of 1980 (Pub. L. 96-354; 94 Stat. 1165 (5 U.S.C. 60 et. seq.)) and it has been determined that it will not have significant economic impact on a substantial number of small businesses, small governments, or small organizations.

C. Paperwork Reduction Act

The Office of Management and Budget (OMB) has approved the information collection requirements contained in this proposed rule under the provisions of the Paperwork Reduction Act of 1980, 44 U.S.C. 3501 *et seq.*, and has assigned OMB control numbers: 2070-0024, 2070-0032, 2070-0040, 2070-0055, 2070-0057, 2070-0060. Comments on these requirements should be submitted to the Office of Information and Regulatory Affairs of OMB, marked "Attention: Desk Officer for EPA." The final rule will respond to any OMB or public comments on the information collection requirements.

List of Subjects in 40 CFR Part 160

Good laboratory practices, Laboratories, Environmental protection, Hazardous materials, Chemicals, Recordkeeping and reporting requirements.

Dated: December 8, 1987.

Lee M. Thomas,

Administrator.

Therefore, it is proposed that 40 CFR Part 160 be amended as follows:

PART 160--[AMENDED]

1. The authority citation for Part 160 continues to read as follows:

Authority: 7 U.S.C. 136a, 136c, 136d, 136f, 136j, 136t, 136v, 136w; 21 U.S.C. 346a, 348, 371, Reorganization Plan No. 3 of 1970.

2. In § 160.3, by removing the alphabetical paragraph designations in paragraphs (a) through (q); by revising the definitions for "Control substance," "Study," and "Test system"; by replacing the term "Test substance or mixture" with the term "Test substance"; and by alphabetically inserting definitions for "Carrier", "Experimental start date", "Experimental termination date", "Reference Substance", "Study completion date", "Study initiation date", and "Vehicle", to read as follows:

§ 160.3 Definitions.

* * * * *

"Carrier" means any material (e.g., feed, water, soil, nutrient media) with which the test substance is combined for administration to test organisms.

"Control substance" means any chemical substance or mixture or any other material other than a test substance, feed, or water that is administered to the test system in the course of study for the purpose of establishing a basis for comparison with the test substance for no-effect levels.

* * * * *

"Experimental start date" means the first date the test substance is applied to the test system.

"Experimental termination date" means the last date on which data are collected directly from the study.

* * * * *

"Reference substance" means any chemical substance or mixture or material other than a test substance, feed, or water that is administered to or used in analyzing the test system in the course of a study for purposes of establishing a basis for comparison with the test substance for known effect levels.

* * * * *

"Study" means any experiment in which a test substance is studied in a test system under laboratory conditions or in the environment to determine or help predict its effects, metabolism, product performance (efficacy as required by 40 CFR 158.160), environmental and chemical fate, persistence and residue, or other characteristics in humans, other living organisms, or media. The term does not include basic exploratory studies carried out to determine whether a test substance has any potential utility.

"Study completion date" means the date the final report is signed by the study director.

* * * * *

"Study initiation date" means the date the protocol is signed by the study director.

"Test substance" means a substance or mixture administered or added to a test system in a study, which substance or mixture:

(1) Is the subject of an application for a research or marketing permit supported by the study, or is the contemplated subject of such an application; or

(2) Is an ingredient, impurity, degradation product, metabolite, or radioactive isotope of a substance described by paragraph (1) of this definition, or some other substance related to a substance described by that paragraph, which is used in the study to assist in characterizing the toxicity, metabolism, or other characteristics of a substance described by that paragraph.

"Test system" means any animal, plant, microorganism, chemical or physical matrix (e.g., soil or water), or subparts thereof, to which the test or control substance is administered or added for study. "Test system" also includes appropriate groups or components of the system not treated with the test, control, or reference substance.

* * * * *

"Vehicle" means any agent which facilitates the mixture, dispersion, or solubilization of a test substance with a carrier.

3. In § 160.29, by revising paragraphs (d), (e), and (f) to read as follows:

§ 160.29 Personnel.

* * * * *

(d) Personnel shall take necessary personal sanitation and health precautions designed to avoid contamination of test, control, and reference substances and test systems.

(e) Personnel engaged in a study shall wear clothing appropriate for the duties they perform. Such clothing shall be changed as often as necessary to prevent microbiological, radiological, or chemical contamination of test systems and test, control, and reference substances.

(f) Any individual found at any time to have an illness that may adversely affect the quality and integrity of the study shall be excluded from direct contact with test systems, and test, control, and reference substances, and any other operation or function that may adversely effect the study until the condition is corrected. All personnel shall be instructed to report to their immediate supervisors any health or medical conditions that may reasonably be considered to have an adverse effect on a study.

4. In § 160.31, by revising paragraph (b) to read as follows:

§ 160.31 Testing facility management.

* * * * *

(b) Replace the study director promptly if it becomes necessary to do so during the conduct of a study.

* * * * *

5. In § 160.35, by revising paragraphs (a) and (b) (1) and (3) and removing paragraph (e) to read as follows:

§ 160.35 Quality assurance unit.

(a) A testing facility shall have a quality assurance unit which shall be responsible for monitoring each study to assure management that the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with the regulations in this part. For any given study, the quality assurance unit shall be entirely separate from and independent of the personnel engaged in the direction and conduct of that study.

(b) The quality assurance unit shall:

(1) Maintain a copy of a master schedule sheet of all studies conducted at the testing facility indexed by test substance and containing the test system, nature of study, date study was

initiated, current status of each study, identity of the sponsor, and name of the study director.

* * * * *

(3) Inspect each study at intervals adequate to ensure the integrity of the study and maintain written and properly signed records of each periodic inspection showing the date of the inspection, the study inspected, the phase or segment of the study inspected, the person performing the inspection, findings and problems, action recommended and taken to resolve existing problems, and any scheduled date for reinspection. Any problems which are likely to affect study integrity found during the course of an inspection shall be brought to the attention of the study director and management immediately.

* * * * *

6. By revising § 160.41 to read as follows:

§ 160.41 General.

Each testing facility shall be of suitable size and construction to facilitate the proper conduct of studies. Testing facilities which are not located within an indoor controlled environment shall be of suitable location to facilitate the proper conduct of studies. Testing facilities shall be designed so that there is a degree of separation that will prevent any function or activity from having an adverse effect on the study.

7. By revising § 160.43 to read as follows:

§ 160.43 Test system care facilities.

(a) A testing facility shall have a sufficient number of animal rooms or other test system areas, as needed, to ensure: proper separation of species or test systems, isolation of individual projects, quarantine or isolation of animals or other test systems, and routine or specialized housing of animals or other test systems.

(1) In tests with plants or aquatic animals, proper separation of species can be accomplished within a room or area by housing them separately in different chambers or aquaria. Separation of species is unnecessary where the protocol specifies the simultaneous exposure of two or more species in the same chamber, aquarium, or housing unit.

(2) Aquatic toxicity tests for individual projects shall be isolated to the extent necessary to prevent cross-contamination of different chemicals used in different tests.

(b) A testing facility shall have a number of animal rooms or other test system areas separate from those described in paragraph (a) of this section to ensure isolation of studies being done with test systems or test, control, and reference substances known to be biohazardous, including volatile substances, aerosols, radioactive materials, and infectious agents.

(c) Separate areas shall be provided, as appropriate, for the diagnosis, treatment, and control of laboratory test system diseases. These areas shall provide effective isolation for the housing of test systems either known or suspected of being diseased, or of being carriers of disease, from other test systems.

(d) Facilities shall have proper provisions for collection and disposal of contaminated water, soil, or other spent materials. When animals are housed, facilities shall exist for the collection and disposal of all animal waste and refuse or for safe sanitary storage of waste before removal from the testing facility. Disposal facilities shall be so provided and operated as to minimize vermin infestation, odors, disease hazards, and environmental contamination.

(e) Facilities shall have provisions to regulate environmental conditions (e.g., temperature, humidity, photoperiod) as specified in the protocol.

(f) For marine test organisms, an adequate supply of clean sea water or artificial sea water (prepared from deionized or distilled water and sea salt mixture) shall be available. The ranges of composition shall be as specified in the protocol.

(g) For freshwater organisms, an adequate supply of clean water of the appropriate hardness, pH, and temperature, and free of contaminants capable of interfering with the study, shall be available as specified in the protocol.

(h) For plants, an adequate supply of soil of the appropriate composition, as specified in the protocol, shall be available as needed.

8. By revising § 160.45 to read as follows:

§ 160.45 Test system supply facilities.

(a) There shall be storage areas, as needed, for feed, nutrients, soils, bedding, supplies, and equipment. Storage areas for feed nutrients, soils, and bedding shall be separated from areas housing the test systems and shall be protected against infestation or contamination. Perishable supplies shall be preserved by appropriate means.

(b) When appropriate, plant supply facilities shall be provided. These include:

(1) Facilities, as specified in the protocol, for holding, culturing, and maintaining algae and aquatic plants.

(2) Facilities, as specified in the protocol, for plant growth (e.g., greenhouses, growth chambers, light banks).

(c) When appropriate, facilities for aquatic animal tests shall be provided. These include aquaria, holding tanks, ponds, and ancillary equipment, as specified in the protocol.

9. By revising § 160.47 to read as follows:

§ 160.47 Facilities for handling test, control, and reference substances.

(a) As necessary to prevent contamination or mixups, there shall be separate areas for:

(1) Receipt and storage of the test, control, and reference substances.

(2) Mixing of the test, control, and reference substances with a carrier, e.g., feed.

(3) Storage of the test, control, and reference substance mixtures.

(b) Storage areas for test, control, and/or reference substance and for test, control, and/or reference mixtures shall be separate from areas housing the test systems and shall be adequate to preserve the identity, strength, purity, and stability of the substances and mixtures.

10. By revising § 160.49 to read as follows:

§ 160.49 Laboratory operation areas.

Separate laboratory space and other space shall be provided, as needed, for the performance of the routine and specialized procedures required by studies.

§ 160.53 [Removed]

11. By removing § 160.53 *Administrative and personnel facilities.*

12. By revising § 160.61 to read as follows:

§ 160.61 Equipment design.

Equipment used in the generation, measurement, or assessment of data and equipment used for facility environmental control shall be of appropriate design and adequate capacity to function according to protocol and shall be suitably located for operation, inspection, cleaning, and maintenance.

13. In § 160.63, by revising paragraph (b) to read as follows:

§ 160.63 Maintenance and calibration of equipment.

* * * * *

(b) The written standard operating procedures required under

§ 160.81(b)(11) shall set forth in sufficient detail the methods, materials, and schedules to be used in the routine inspection, cleaning, maintenance, testing, calibration, and/or standardization of equipment, and shall specify, when appropriate, remedial action to be taken in the event of failure or malfunction of equipment. The written standard operating procedures shall designate the person responsible for the performance of each operation.

* * * * *

14. In § 160.81, by revising paragraphs (b) (1), (2), (3), (5), (6), (7), and (12) and (c) to read as follows:

§ 160.81 Standard operating procedures.

* * * * *

(b) * * *

(1) Test system room preparation.

(2) Test system care.

(3) Receipt, identification, storage, handling, mixing, and method of sampling of the test, control, and reference substances.

* * * * *

(5) Laboratory or other tests.

(6) Handling of test systems found moribund or dead during study.

(7) Necropsy of test systems or postmortem examination of test systems.

* * * * *

(12) Transfer, proper placement, and identification of test systems.

(c) Each laboratory or other study area shall have immediately available manuals and standard operating procedures relative to the laboratory or field procedures being performed. Published literature may be used as a supplement to standard operating procedures.

* * * * *

15. By revising § 160.90 to read as follows:

§ 160.90 Animal and other test system care.

(a) There shall be standard operating procedures for the housing, feeding, handling, and care of animals and other test systems.

(b) All newly received test systems from outside sources shall be isolated and their health status or appropriateness for the study evaluated. This evaluation shall be in accordance with acceptable veterinary medical practice or scientific practice.

(c) At the initiation of a study, test systems shall be free of any disease or condition that might interfere with the purpose or conduct of the study. If during the course of the study, the test systems contract such a disease or condition, the diseased test systems should be isolated, if necessary. These test systems may be treated for disease or signs of disease provided that such treatment does not interfere with the study. The diagnosis, authorization of treatment, description of treatment, and each date of treatment shall be documented and shall be retained.

(d) Warm-blooded animals, adult reptiles, and adult terrestrial amphibians used in laboratory procedures that require manipulations and observations over an extended period of time or in studies that require these test systems to be removed from and returned to their test system-housing units for any reason (e.g., cage cleaning, treatment, etc.), shall receive appropriate identification (e.g., tattoo, toe clip, color code, ear tag, ear punch, etc.). All information needed to specifically identify each test system within the test system-housing unit shall appear on the outside of that unit. Suckling mammals and juvenile birds are excluded from the requirement of individual identification unless otherwise specified in the protocol.

(e) Except as specified in paragraph (e)(1) of this section, test systems of different species shall be housed in separate rooms when necessary. Test systems of the same species, but used in different studies, should not ordinarily be housed in the same room when inadvertent exposure to test, control, or reference substances or test system mixup could affect the outcome of either study. If such mixed housing is necessary, adequate differentiation by space and identification shall be made.

(1) Plants, invertebrate animals, aquatic vertebrate animals, and organisms that may be used in multispecies tests need not be housed in separate rooms, provided that they are adequately segregated to avoid mixup and cross contamination.

(2) [Reserved]

(f) Cages, racks, pens, enclosures, aquaria, holding tanks, ponds, growth chambers, and other holding, rearing and breeding areas, and accessory equipment, shall be cleaned and sanitized at appropriate intervals.

(g) Feed, soil, and water used for the test systems shall be analyzed periodically to ensure that contaminants known to be capable of interfering with the study and reasonably expected to be present in such feed, soil, or water are not present at levels above those specified in the protocol. Documentation of such analyses shall be maintained as raw data.

(h) Bedding used in animal cages or pens shall not interfere with the purpose or conduct of the study and shall be changed as often as necessary to keep the animals dry and clean.

(i) If any pest control materials are used, the use shall be documented. Cleaning and pest control materials that interfere with the study shall not be used.

(j) All plant and animal test organisms shall be acclimatized, prior to their use in an experiment, to the environmental conditions of the test.

Subpart F—Test, Control, and Reference Substances

16. By revising the heading for Subpart F to read as set forth above.

17. By revising § 160.105 to read as follows:

§ 160.105 Test, control, and reference substance characterization.

(a) The identity, strength, purity, and composition, or other characteristics which will appropriately define the test, control, or reference substance shall be determined for each batch and shall be documented before its use in an experiment. Methods of synthesis, fabrication, or derivation of the test, control, or reference substance shall be documented by the sponsor or the testing facility.

(b) The stability and, when relevant to the conduct of the experiment, the solubility of each test, control, or reference substance shall be determined by the testing facility or by the sponsor before the experimental start date. Where periodic analysis of each batch is required by the protocol, there shall be written standard operating procedures that shall be followed.

(c) Each storage container for a test, control, or reference substance shall be labeled by name, chemical abstracts service number (CAS) or code number, batch number, expiration date, if any, and, where appropriate, storage conditions necessary to maintain the identity, strength, purity, and composition of the test, control, or reference substance. Storage containers shall be assigned to a particular test substance for the duration of the study.

(d) For studies of more than 4 weeks duration, reserve samples from each batch of test, control, and reference substances shall be retained for the period of time provided by § 160.195.

(e) The stability of test, control, and reference substances under test conditions shall be known for all studies.

18. In § 160.107, by revising the section heading and introductory text to read as follows:

§ 160.107 Test, control, and reference substance handling.

Procedures shall be established for a system for the handling of the test, control, and reference substances to ensure that:

* * * * *

19. By revising § 160.113 to read as follows:

§ 160.113 Mixtures of substances with carriers.

(a) For each test, control, or reference substance that is mixed with a carrier, tests by appropriate analytical methods shall be conducted:

(1) To determine the uniformity of the mixture and to determine, periodically, the concentration of the test, control, or reference substance in the mixture.

(2) To determine the stability and, when relevant to the conduct of the experiment, the solubility of the test, control, or reference substance in the mixture, before the experimental start date. Determination of the stability and solubility of the test, control, or reference substance in the mixture shall be done under the environmental conditions specified in the protocol and as required by the conditions of the experiment. Where periodic analysis of the mixture is required by the protocol, there shall be written standard operating procedures that shall be followed.

(b) Where any of the components of the test, control, or reference substance carrier mixture has an expiration date, that date shall be clearly shown on the container. If more than one component has an expiration date, the earliest date shall be shown.

(c) If a vehicle is used to facilitate the mixing of a test substance with a carrier, assurance shall be provided that the vehicle does not interfere with the integrity of the test.

20. In § 160.120, by revising paragraph (a) to read as follows:

§ 160.120 Protocol.

(a) Each study shall have an approved written protocol that clearly indicates the objectives and all methods for the conduct of the study. The protocol shall contain but shall not necessarily be limited to the following information:

(1) A descriptive title and statement of the purpose of the study.

(2) Identification of the test, control, and reference substance by name, chemical abstracts service (CAS) number or code number.

(3) The name and address of the sponsor and the name and address of the testing facility at which the study is being conducted.

(4) The proposed experimental start and termination dates.

(5) Justification for selection of the test system.

(6) Where applicable, the number, body weight, sex, source of supply, species, strain, substrain, and age of the test system.

(7) The procedure for identification of the test system.

(8) A description of the experimental design, including methods for the control of bias.

(9) Where applicable, a description and/or identification of the diet used in the study as well as solvents, emulsifiers and/or other materials used to solubilize or suspend the test, control, or reference substances before mixing with the carrier. The description shall include specifications for acceptable levels of contaminants that are reasonably expected to be present in the dietary materials and are known to be capable of interfering with the purpose or conduct of the study if present at levels greater than established by the specifications.

(10) The route of administration and the reason for its choice.

(11) Each dosage level, expressed in milligrams per kilogram of body or test system weight or other appropriate units, of the test, control, or reference substance to be administered and the method of frequency of administration.

(12) The type and frequency of test analyses, and measurements to be made.

(13) The records to be maintained.

(14) The date of approval of the protocol by the sponsor and the dated signature of the study director.

(15) A statement of the proposed statistical method.

* * * * *

21. In § 160.130, by revising paragraphs (d) and (e) to read as follows:

§ 160.130 Conduct of a study.

* * * * *

(d) In animal studies where histopathology is required, records of gross findings for a specimen from postmortem observations shall be available to a pathologist when examining that specimen histopathologically.

(e) All data generated during the conduct of a study, except those that are generated by automated data collection systems, shall be recorded directly, promptly, and legibly in ink. All data entries shall be dated on the day of entry and signed or initialed by the person entering the data. Any change in entries shall be made so as not to obscure the original entry, shall indicate the reason for such change, and shall be dated and signed or identified at the time of the change. In automated data collection systems, the individual responsible for direct data input shall be identified at the time of data input. Any change in automated data entries shall be made so as not to obscure the original entry, shall indicate the reason for change, shall be dated, and the responsible individual shall be identified.

22. By adding § 160.135 to read as follows:

§ 160.135 Physical and chemical characterization studies.

(a) Except as provided in paragraph (b) of this section, the following provisions shall not apply to studies designed to determine physical and chemical characteristics of a test, control, or reference substance:

§ 160.31 (c), (d), and (g)
§ 160.35 (b) and (c)
§ 160.43
§ 160.45
§ 160.47
§ 160.49
§ 160.81(b) (1), (2), (6) through (9), and (12)
§ 160.90
§ 160.105 (a) through (d)
§ 160.113
§ 160.120(a) (5) through (12), and (15)
§ 160.185(a) (5) through (8), (10), (12), and (14)
§ 160.195 (c) and (d)

(b) The exemptions provided in paragraph (a) of this section shall not apply to physical/chemical characterization studies designed to determine stability, solubility, octanol water partition coefficient, volatility, and persistence (such as biodegradation, photodegradation, and chemical degradation studies), and such studies shall be conducted in accordance with this part.

23. In § 160.185, by revising paragraphs (a) (4) and (5) to read as follows:

§ 160.185 Reporting of study results.

(a) * * *

(4) The test, control, and reference substances identified by name, chemical abstracts service (CAS) number or code number, strength, purity, and composition, or other appropriate characteristics.

(5) Stability and, when relevant to the conduct of the experiment, the solubility of the test, control, and reference substances under the conditions of administration.

* * * * *

24. In § 160.190, by revising paragraphs (a) and (e) to read as follows:

§ 160.190 Storage and retrieval of records and data.

(a) All raw data, documentation, records, protocols, specimens, and final reports generated as a result of a study shall be retained. Specimens obtained from mutagenicity tests, specimens of soil, water, and plants, and wet specimens of blood, urine, feces, and biological fluids, do not need to be retained beyond quality assurance. Correspondence and other documents relating to interpretation and evaluation of data, other than those documents contained in the final report, also shall be retained.

* * * * *

(e) Material retained or referred to in the archives shall be indexed to permit expedient retrieval.

25. In § 160.195, by revising paragraph (c) and adding paragraph (i) to read as follows:

§ 160.195 Retention of records.

* * * * *

(c) Wet specimens, samples of test, control, or reference substances, and specially prepared material which are relatively fragile and differ markedly in stability and quality during storage, shall be retained only as long the quality of the preparation affords evaluation. Specimens obtained from mutagenicity tests, specimens of soil, water, and plants, and wet specimens of blood, urine, feces, biological fluids, do not need to be retained beyond quality assurance review. In no case shall retention be required for longer periods than those set forth in paragraph (b) of this section.

* * * * *

(i) Records required by this part may be retained either as original records or as true copies such as photocopies, microfilm, microfiche, or other accurate reproductions of the original records.

[FR Doc. 87-29511 Filed 12-24-87; 8:45 am]
BILLING CODE 6560-50-M

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40 CFR Part 792

[OPTS-46016; FRL-3245-6]

Toxic Substances Control Act (TSCA); Good Laboratory Practice Standards

AGENCY: Environmental Protection Agency (EPA).

ACTION: Proposed rule.

SUMMARY: EPA is proposing to amend the TSCA Good Laboratory Practice (GLP) Standards to incorporate many of the changes made by the Food and Drug Administration (FDA) to its GLP regulations and to expand the scope of the TSCA GLP standards to apply to testing conducted in the field under TSCA. EPA is proposing this amendment in order to ensure the quality and integrity of data generated from such studies.

DATE: Submit written comments on or before March 28, 1988.

ADDRESS: Submit written comments, identified by the document control number (OPTS-46016), in triplicate to: TSCA Public Information Office (TS-793), Office of Pesticides and Toxic Substances, Environmental Protection Agency, Rm. NE-G004, 401 M St., SW., Washington, DC 20460.

The public record supporting this action is available for inspection at the above address from 8 a.m. to 4 p.m., Monday through Friday, except legal holidays.

FOR FURTHER INFORMATION CONTACT: Edward A. Klein, Director, TSCA Assistance Office (TS-799), Office of Toxic Substances, Rm. E-543, 401 M St., SW., Washington, DC 20460 (202) 554-1404.

SUPPLEMENTARY INFORMATION: Following is an index to the remainder of this preamble:

I. Introduction

A. Legal Authority

On November 29, 1983 (48 FR 53922), EPA promulgated the GLP standards under the authority of TSCA section 4 (90 Stat. 2006, 15 U.S.C. 2603). Section 4(a) of TSCA authorizes the EPA Administrator to require, by rule, that manufacturers (including importers) and processors of identified chemical substances and mixtures test such chemicals if certain findings are made. Section 4(b)(1) of TSCA specifies that each test rule shall include standards for the development of test data. These standards are defined in section 3(12) of TSCA to mean a prescription of—

(A) the—

(i) health and environmental effects, and

(ii) information relating to the toxicity, persistence, and other characteristics which affect health and the environment, for which test data for a chemical substance or mixture are to be developed and any analysis that is to be performed on such data, and

(B) to the extent necessary to assure that data respecting such effects and characteristics are reliable and adequate—

(i) the manner in which such data are to be developed,

(ii) the specification of any test protocol or methodology to be employed in the development of such data, and

(iii) such other requirements as are necessary to provide such assurance.

In summary, the specific authority to issue the GLP standards is provided by section 4(b)(1) of TSCA, which is further explained by the definitions in sections 3(12)(B)(i) and 3(12)(B)(iii).

In addition, the Agency also requires sponsors to utilize these GLP standards when conducting testing under TSCA section 4 testing consent agreements and will include provisions to adhere to these GLP standards in those agreements (see 40 CFR 790.60(a)(7)). Also, it is the Agency's policy that all data developed as a result of rules or orders under section 5 of TSCA should be in accordance with the GLP standards. If data developed under section 5 of TSCA are not generated in accordance with the GLP standards, the Agency may elect to consider such data insufficient to evaluate the health effects, environmental effects, and fate of the chemical.

B. Background

EPA originally published enforceable TSCA Good Laboratory Practice Standards in the **Federal Register** of November 29, 1983 (48 FR 53922), which were codified as 40 CFR Part 792. At the same time, EPA published GLP standards applicable to testing under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA, 48 FR 53963, 40 CFR Part 160). These regulations were promulgated in response to investigations by EPA and FDA during the mid-1970s which revealed that some studies submitted to the Agencies had not been conducted in accordance with acceptable laboratory practices. Some studies had been conducted so poorly that the resulting data could not be relied upon in EPA's regulatory decisionmaking process. For instance, some studies had been submitted which did not adhere to specified protocols, were conducted by underqualified personnel and supervisors, or were not adequately monitored by study sponsors. In some cases results were selectively reported, underreported, or fraudulently reported. In addition, it was discovered that some testing facilities displayed poor animal care procedures and inadequate recordkeeping techniques. The TSCA GLP standards specify minimum practices and

procedures which must be followed in order to ensure the quality and integrity of data submitted in accordance with TSCA section 4 requirements. The 1983 TSCA GLP standards also established a policy that persons should comply with the GLP standards when submitting data in response to rules and orders issued under section 5 of TSCA, and when submitting data to the Agency voluntarily.

When EPA published its final TSCA and FIFRA GLP standards in the **Federal Register** of November 29, 1983, the Agency sought to harmonize the requirements and language with those regulations promulgated by the FDA in the **Federal Register** of December 22, 1978 (43 FR 60013), and codified as 21 CFR Part 58. Differences between the two Agencies' current GLP regulations exist only to the extent necessary to reflect the Agencies different statutory responsibilities under TSCA, FIFRA, and the Federal Food, Drug, and Cosmetic Act (FFDCA). Similar to the FDA GLP regulations, the FIFRA and TSCA GLPs delineate standards for studies designed to determine the health effects of a test substance; however, the TSCA GLPs also contain provisions related to environmental testing (i.e., ecological effects and chemical fate).

Compliance with EPA's GLP regulations has been monitored through a program of laboratory inspections and study audits coordinated between EPA and FDA. Under an Interagency Agreement originating in 1978, FDA carries out inspections at laboratories which conduct health effects testing. EPA primarily performs laboratory inspections and data audits for environmental studies.

After a thorough review of its GLP regulations and compliance program, FDA concluded that some of the provisions of the GLPs needed to be clarified, amended, or deleted in order to reduce the regulatory burden on testing facilities. Accordingly, FDA proposed revisions to its GLP regulations in the **Federal Register** of October 24, 1984 (49 FR 43530), which were intended to simplify the regulation without compromising study integrity. FDA's proposed revision has recently been published as a final rule in the **Federal Register** of September 4, 1987 (52 FR 33768).

EPA agrees with FDA that many provisions of the GLP regulations can be streamlined without compromising the goals of the GLPs. Therefore, EPA is proposing to amend the TSCA GLP standards to incorporate many of the changes recently made by FDA to its GLP regulations. In addition, EPA is proposing to expand the scope of the TSCA GLPs to cover testing wherever it is conducted (e.g., field testing). In another notice in this **Federal Register** EPA is proposing similar changes to the FIFRA GLP standards.

C. Consistency With FDA GLP Regulations

It is EPA's policy to minimize the regulatory burden on the public which might arise from conflicting requirements which could be promulgated under different regulatory authorities. In keeping with this policy, the final 1983 TSCA GLP Standards, 40 CFR Part 792, followed the format and, with few exceptions, the wording of FDA's final GLP regulations, 21 CFR Part 58. Differences between the EPA and FDA GLP regulations were based upon varying needs and responsibilities under each Agency's regulatory statutes. This proposed revision to the TSCA GLP standards follows this same policy by conforming to many of the changes FDA made to its GLP regulations, published in the **Federal Register** of September 4, 1987 (52 FR 33768). EPA has varied from FDA's revised GLP regulations only when necessary due to EPA's statutory responsibilities. The most significant differences between the EPA proposal and the revised FDA GLP regulations are the scope of the testing and test systems affected.

As in the current TSCA Good Laboratory Practice Standards, the proposed revisions to the TSCA GLP standards vary from the FDA GLPs in that the TSCA GLPs incorporate GLP provisions for environmental testing (EPA is proposing that the FIFRA GLPs extend to environmental studies as well). Environmental studies include ecological effects and chemical fate studies. Ecological effects studies are those performed for development of information on nonhuman toxicity and potential ecological impact of chemicals and their degradation products. Chemical fate studies are studies performed to characterize physical, chemical, and persistence properties of a substance in order to evaluate the transport and transformation of the substance in the environment.

To ensure the quality and integrity of all data generated from environmental studies, the current TSCA GLP standards contain requirements within 40 CFR Part 792 Subpart L applicable to testing plants, microbial organisms, aquatic organisms, amphibians, reptiles, and birds, where appropriate. These requirements include provisions for care, care facilities, and supply facilities for the various test systems used in environmental testing. As a means of simplifying the regulations, EPA is proposing that the requirements currently found within Subpart L be merged into Subparts A through J of the TSCA GLPs. Accordingly, it is proposed that current § 792.43 *Animal care facilities*, § 792.45 *Animal supply facilities*, and § 792.90 *Animal care* incorporate the provisions relating to the care of test systems, care facilities, and supply facilities from § 792.228 in Subpart L. The expanded sections are retitled in the proposed revision as follows: § 792.43 *Test system care facilities*, § 792.45 *Test system supply facilities*, and § 792.90 *Animal and other test system care*. Further, in most instances, EPA is proposing to replace the term "animal," currently used in the EPA and FDA GLP regulations, with the broader term "test system." Specifically, this change is proposed in §§ 792.43, 792.45, 792.81, 792.90, and 792.120. These proposed changes are further discussed in Unit I.D. of this preamble.

EPA's proposed TSCA GLP standards also vary from FDA's in their coverage of testing conducted in the field. To ensure the quality and integrity of data submitted to the Agency, EPA believes that GLP standards must apply whenever data collection occurs. Because many of the test data required by EPA are developed in the field, or more accurately in outdoor laboratories (i.e., ground water studies, air monitoring studies, degradation in soil, etc.), EPA is proposing to include field testing within the scope of these regulations.

The remaining differences between the EPA and FDA GLPs are described in the preamble to this proposed rule and the preamble to the TSCA Good Laboratory Practice Standards, published in the **Federal Register** of November 29, 1983 (48 FR 53922). EPA has coordinated this proposal with FDA and has considered comments received on the proposal to amend the FDA GLP regulations (October 29, 1984; 49 FR 43530).

D. Proposed Changes to the TSCA GLP Regulations

1. *Section 792.1 Scope.* EPA proposes to amend § 792.1 to reflect the Agency's option of entering into testing consent agreements in lieu of a test rule under section 4 of TSCA. Consistently, the term "testing consent agreement" has been added to the definition of "test substance" in proposed § 792.3, and has been added in proposed §§ 792.12 and 792.17.

2. *Section 792.3 Definitions.* a. EPA proposes that the definition of the term "carrier" be moved from § 792.226(b) to § 792.3. As stated in Unit I.C. of this

preamble, EPA is proposing to delete Subpart L and include all the provisions of Subpart L within Subparts A through J of the TSCA GLP standards. Therefore, EPA proposes to define the term "carrier" in § 792.3 to mean any material, such as feed, water, soil, nutrient material, etc., with which the test substance is combined for administration to test organisms.

b. EPA proposes to conform with the September 4, 1987, FDA GLP regulations by amending the definition of "control substance" to exclude feed and water. EPA agrees with FDA's statement regarding this change (52 FR 33769; September 4, 1987) that "the term control [substance] should be reserved for the discrete substances/articles, and vehicles other than feed and water administered to groups of the test system to provide a basis of comparison with the test [substance]."

FDA contends that, under the current definition of "control substance," because the control group of a test system provides the basis for comparison with a test substance, any substance administered to the control group is considered a control substance. This means that feed and water given to the control group of a study are considered a control substance. For instance, in studies in which the test substance or mixture is administered to the test system orally, through feed or drinking water, gavage, or injection, the feed or water is considered a control substance. As a control substance, the feed or water is subject to § 792.105(a) for substance characterization, § 792.105(b) for testing for stability and solubility, § 792.105(c) for requirements for appropriate storage, § 792.105(d) for retention of reserve samples, and § 792.107 for documentation of receipt and distribution of each batch. EPA agrees with FDA that placing these requirements on the use of feed and water as a control substance in control groups unnecessarily burdens the regulated community and is not essential for ensuring the quality and integrity of the data generated by a study.

However, under 40 CFR Part 792, feed and water used as a carrier for the test and control substances or mixtures are still covered by the applicable sections for the testing and storage of test, control, and reference substances and mixtures. For example, § 792.31(e) requires testing facility management to ensure that materials are available as scheduled; § 792.45 requires that test system supply facilities shall be provided to ensure proper feed storage; § 792.81(b)(2) requires Standard Operating Procedures (SOP) for test system care, including nutrition; § 792.90(g) requires periodic analysis of feed and water to ensure that contaminants which would interfere with the study are not present; § 792.120(a)(9) requires the protocol to describe and/or identify the diet used in the study, including the level of contaminants expected in the dietary materials.

c. EPA also proposes to modify the definition of "control substance" by adding the phrase "for no effect levels." This addition to the definition is being proposed merely to clarify the difference between the term "reference substance" and "control substance." While a control substance is used to determine a baseline comparison for no effect levels, a reference substance is used to determine a baseline comparison to an established effect level.

d. EPA proposes to add and define the terms "experimental start date" and "experimental termination date." "Experimental start date" is proposed to mean the first date the test substance is applied to the test system. Under this definition, as of the experimental start date: (1) Under proposed § 792.105(b), the stability and, if important to the conduct of the experiment, the solubility of the test, control, and reference substance would have to be determined; (2) under proposed § 792.113(a)(2), the stability and, when important to the conduct of the experiment, the solubility of the test, control, and reference substance in the mixture would have to be determined and; (3) under proposed § 792.120(a)(4), the proposed experimental start date would appear in the protocol.

EPA proposes that "experimental termination date" be defined as the last date on which data are collected directly from the study. Under § 792.120(a)(4), as proposed, EPA would require the proposed experimental termination date to appear in the protocol. EPA considers histopathology after scheduled terminal animal sacrifice to be carried out before the experimental termination date.

Experimental start and termination dates would be expressed as the actual calendar dates, not just time-line increments. Therefore, when determining the proposed experimental start and termination dates, as would be required by proposed § 792.120(a)(4), the submitter should consider any lag time relating to protocol approval and laboratory contracting.

e. EPA proposes to add and define the term "reference substance". This term is currently defined in § 792.226(f) to mean any chemical substance or mixture or material other than a test substance that is administered to or used in analyzing the test system in the course of a study for purposes of establishing a basis for comparison with the test substance. EPA proposes to add the phrase "for known effect levels" to this definition to more clearly distinguish the terms "reference substance" and "control substance" (see discussion of the term "control substance" in Unit I.D. of this preamble).

Consistent with the Agency's proposal to merge the provisions of Subpart L into Subparts A through J, all the requirements provided for test and control substances are being proposed to apply to "reference substances." Accordingly, the term "reference substance" has been added wherever the term "test and control substance" appears in these regulations. Specifically, it is proposed that the term "reference substance" be added to § 792.29 (d) through (f); § 792.43(b); § 792.47(a) (1) through (3) and (b); § 792.81(b)(3); § 792.90(e); the Subpart F heading; § 792.105 (a) through (e); § 792.107; § 792.113 (a) and (b); § 792.120(a) (2), (9), and (11); § 792.185(a) (4) and (5); and § 792.195(c).

f. EPA proposes to amend the definition of "sponsor" by replacing the term "negotiated testing agreement" with the term "testing consent agreement." This proposal reflects the Agency's option of entering into a section 4 testing consent agreement in lieu of a test rule promulgated under section 4 of TSCA.

g. EPA proposes to broaden the definition of the term "study" to be consistent with the scope of testing that may be submitted under TSCA sections 4 and 5.

EPA is proposing to delete the phrase "in vivo or in vitro" from the definition of "study." The Agency still intends the requirements of these regulations to apply to "in vivo and in vitro" experiments. However, since the Agency intends these regulations to apply to all studies required to be developed under TSCA, including those conducted in the field, EPA believes that the phrase "in vivo or in vitro" in the current definition of "study" is too limiting.

Further, EPA is proposing to delete the term "prospectively" from the definition of "study." In this way, epidemiological studies, which could be "retrospective," will be required to be presented to the Agency in accordance with the GLP standards. EPA recognizes that data used in an epidemiological study may not have been generated in conformance

with the TSCA GLP standards, however, it is EPA's contention that the epidemiological study itself can be conducted and submitted to the Agency in accordance with the GLPs.

EPA is also proposing to delete from the current definition of "study" the following sentence: "The term does not include studies utilizing human subjects or clinical studies or field trials in animals." Again, this change is consistent with EPA's intention that all studies follow GLPs which are required to be conducted under TSCA.

h. EPA proposes to incorporate the FDA definitions for "study completion date" and "study initiation date" into the TSCA GLP standards in § 792.3. "Study completion date" is proposed to mean the date the final report is signed by the study director. EPA advises that the phrase "close of the study" as used in § 792.33(f) refers to the "study completion date." Therefore, as of that date: (1) Under § 792.33(f), the study director must ensure that all raw data, documentation, protocols, specimens, and final reports are transferred to the archives; and (2) after this date under § 792.185(c), corrections or additions to the final report must be in the form of an amendment by the study director under the procedures specified in that section.

EPA proposes to define "study initiation date" as the date the protocol is signed by the study director. EPA advises that the phrase "study is initiated" as used in § 792.31(a), and the phrase "study was initiated" as used in § 792.35(b)(1) refer to the "study initiation date." Therefore, as of the study initiation date: (1) Under § 792.31(a), the testing facility management would designate a study director; (2) under § 792.35(b)(1), the study would be entered on the master schedule sheet by the quality assurance unit; and (3) under § 792.120(b), after this date all changes or revisions in the protocol would be documented, signed by the study director, and dated. EPA also expects that as of the study initiation date, under § 792.31(e), the testing facility management would have ensured that personnel resources, facilities, equipment, material, and methodologies are available as scheduled.

i. EPA proposes to replace the term "test substance or mixture" with the term "test substance." This is an editorial change which makes usage consistent in the GLP standards. The term "test substance" is proposed to be defined to include mixtures.

j. EPA proposes to incorporate the definition of the term "test system" currently found at § 792.226(a) into the definition of "test system" currently found at § 792.3(p). Therefore, the proposed definition of "test system" in proposed § 792.3 will include chemical or physical matrices (e.g., soil or water).

k. EPA proposes to incorporate the term "vehicle" currently found in § 792.226(g) into § 792.3 *Definitions.*

3. *Section 792.31 Testing facility management.* In conformance with the revised FDA GLP regulations, in § 792.31(b), EPA proposes to delete the requirement that the replacement of a study director must be documented as "raw data." EPA agrees with FDA that this requirement is redundant with other provisions of the GLPs. For instance, § 792.35(b)(1) states that the master schedule sheet must contain the name of the study director. As FDA notes (52 FR 33770), any replacement of the study director would be reflected on the master schedule sheet, which is already considered "raw data." Further, § 792.120(b) states that all changes in an approved protocol must be documented and signed by the study director. Replacement of the study director is considered to be a change in the approved protocol.

4. *Section 792.35 Quality assurance unit (QAU).* a. In § 792.35(a), EPA proposes to conform with the revised FDA GLP regulations by substituting the term "which" for the current phrase "composed of one or more individuals who." This change clarifies that EPA does not require the QAU to be a fixed, permanently staffed unit whose only functions are to monitor the quality of a study. The Agency is only concerned that there be a distinct separation of duties between those personnel involved with the conduct or direction of a study and those personnel performing quality assurance on the same study. Therefore, EPA does intend proposed § 792.35(a) to prohibit personnel from performing quality assurance activities on their own study.

b. In § 792.35(b)(1), EPA proposes to delete the requirement that the name of the study sponsor appear on the master schedule sheet. Instead, it is proposed that under § 792.35(b)(1) the sponsor's identity appear on the master schedule sheet. This change is being proposed to be consistent with the FDA's recent revision and to provide the regulated community the option of using an identity code on the master schedule in lieu of the sponsor's name.

EPA agrees with FDA's contention that requiring the sponsor to be identified specifically by name on the master schedule is not essential to fulfill the requirements of the GLPs or the goal of ensuring the quality and integrity of the data generated from the studies. However, while the name of the study sponsor would not be required to appear on the master schedule sheet, this information must be made available to the Agency upon request.

c. As in the revised FDA GLP regulations, EPA is also proposing to delete the requirement in § 792.35(b)(1) that the master schedule sheet contain the status of the final report. EPA agrees with FDA that this requirement is redundant in view of the other information required by § 792.35(b)(1) such as the date the experiment began and the current status of each study.

d. In conformance with the revised FDA GLP regulations, EPA proposes to modify the requirements of § 792.35(b)(3) to provide for inspections of a study on a schedule adequate to ensure the integrity of the study. This section currently specifies that the quality assurance unit must inspect each phase of a study periodically. This section also currently specifies that for studies lasting more than 6 months, quality assurance inspections shall be conducted every 3 months, and for studies lasting less than 6 months, quality assurance inspections shall be conducted at intervals adequate to ensure the integrity of the study.

The proposed changes to this section will allow the QAU the necessary latitude to adjust its monitoring activities to meet the individual problems of each study. EPA agrees with FDA's contention that an inspection of each phase of the study is not necessary to ensure that a study is being conducted properly. However, EPA also agrees with FDA that each study, no matter how short, must be inspected at least once while in process. EPA expects that by allowing the QAU flexibility in designing a reasonable inspection schedule, the goal of ensuring the quality of the study can be best achieved.

e. Consistent with the revised FDA GLPs, EPA is proposing to delete § 792.35(e) in its entirety. Section 792.35(e) currently requires that all quality assurance records be kept in one location at the testing facility. As FDA pointed out in its October 29, 1984, proposed GLP revision, since § 792.190(b) already requires the use of archives for the orderly storage and expedient retrieval of all reports and records, the requirements of § 792.35(e) are not necessary. However, EPA maintains that all reports and records, including those of the QAU, must be easily accessible and made available to EPA and FDA inspectors when requested.

5. *Section 792.41 General.* FDA has deleted from its GLPs the requirement

that the location of each testing facility be suitable to facilitate the proper conduct of studies. However, EPA is proposing that § 792.41 require that testing facilities which are not located within an indoor controlled environment be suitably located to facilitate the proper conduct of studies.

The studies FDA requires are generally conducted within the confines of a traditional indoor laboratory. Because the conditions specified within a protocol can be artificially manipulated within the traditional indoor laboratory, the location of these laboratories is generally not a factor in determining the quality of a study. Therefore, it is not necessary to ensure that a traditional indoor testing facility is suitably located to facilitate the proper conduct of the study.

However, the studies EPA requires are not necessarily conducted within the confines of the traditional indoor scientific laboratory (i.e., field studies, exposure monitoring studies, ecological toxicity studies, etc.). EPA considers any site where testing is undertaken to generate data required by the Agency to be a testing facility. The conditions required by the protocol are not necessarily conducive to artificial manipulation in the field, or other outdoor testing facilities. Therefore, ensuring the suitability of the location of these types of testing facilities is both a valid and necessary part of EPA's GLP Standards.

6. *Section 792.43 Test system care facilities.* a. EPA is proposing to revise the title of § 792.43 from "Animal care facilities" to "Test system care facilities." The proposed heading for § 792.43 more adequately reflects the Agency's intent to specify within the main body of the TSCA GLP Standards the requirements for testing facilities for the care of chemical or physical matrices (*e.g.*, soil or water), plants, and microorganisms, as well as animals. Accordingly, the Agency is proposing to further modify § 792.43 by incorporating the term "test system" when facility requirements should extend beyond "animal" care.

b. Consistent with the Agency's intent to incorporate the environmental testing provisions currently found in Subpart L into Subparts A through J of Part 792, paragraphs (a)(1), (a)(2), (d), (e), (f), (g), and (h) in proposed § 792.43 have been added or modified to incorporate the provisions currently found in § 792.228(b) (1) through (7).

c. EPA proposes to modify § 792.43(a) to allow testing facilities to provide for isolation areas rather than quarantine areas. This change is consistent with the proposal to modify § 792.90(b) to allow "isolation" of newly received animals rather than requiring "quarantine" [See Unit I.D. of this preamble for a discussion of proposed § 792.90(b)].

d. In § 792.43(c), EPA proposes to delete the requirement that separate areas be provided in all cases for the diagnosis, treatment, and control of test system diseases. Instead, it is proposed that such separate areas be provided "as appropriate." This proposal is consistent with the September 4, 1987, revised FDA GLP regulations.

EPA has proposed this modification in order to allow laboratories the option of disposing of diseased animals and other test systems from the experiment without also bearing the expense of maintaining separate areas in testing facilities for diagnosis, treatment, and control of disease. Additionally, EPA recognizes that the diagnosis and treatment requirements of § 792.43(c) may not be appropriate when dealing with such test systems as soil, plants, or microorganisms. However, if the decision is made not to dispose of the test system from the study, then test system care facilities, as specified in proposed § 792.43(c), must be provided.

e. EPA proposes to conform to the revised FDA GLPs by deleting § 792.43(e) in its entirety. Currently, § 792.43(e) requires test system facilities to be designed, constructed, and located so as to minimize disturbances which may interfere with the study. EPA agrees with FDA that this provision is already adequately covered in § 792.41, which requires that facilities be of suitable size construction, and, for outdoor testing facilities, location to facilitate the proper conduct of the study.

7. *Section 792.45 Test system supply facilities.* a. EPA proposes to incorporate the provisions of § 792.228(c) into § 792.45. Therefore, proposed § 792.45 will require that supply facilities necessary for environmental testing be provided when appropriate.

b. Consistent with the proposed expanded scope of this section, EPA is also proposing to retitle § 792.45 from "Animal supply facilities" to "Test system supply facilities."

c. EPA proposes to modify § 792.45 to state "Perishable supplies shall be preserved by appropriate means." This change is being proposed to conform with the revised FDA GLPs and recognizes that there are a variety of acceptable storage and preservation procedures available other than refrigeration. Depending on the stability characteristics of the perishable material, acceptable storage and preservation methods may include desiccation, room temperature-low humidity, and constant temperature-low humidity.

d. EPA also proposes to delete the phrase "or feed" from the last sentence of § 792.45. Both EPA and FDA consider "feed" to be a "supply." Therefore, the use of the word "feed" in § 792.45 is redundant.

8. *Section 792.49 Laboratory operation areas.* a. EPA proposes to conform with FDA's revised GLP regulations by deleting paragraph (b) from § 792.49, adding the phrase "and specialized" after the word "routine" and before the word "procedures," and deleting the qualifying phrase "including specialized areas for performing activities such as aseptic surgery, intensive care, necropsy, histology, radiography, and handling of biohazardous materials."

Paragraphs (a) and (b), as currently worded, describe activities which require that separate laboratory space be provided. As FDA noted in its proposal to modify its corresponding section, the list of activities that currently appears in paragraphs (a) and (b) is not all inclusive and is not essential for the clarity of these sections. Further, by adding the phrase "and specialized," the proposed new paragraph will encompass all activities now listed in paragraphs (a) and (b).

b. In § 792.49, EPA proposes to add the phrase "and other space" after the words "laboratory space" and before the word "shall." As discussed in Unit I.C. of this preamble, this change to § 792.49 is being proposed to reflect that testing does not necessarily take place within the confines of a traditional indoor laboratory. Proposed § 792.49 would require that there be enough space provided to perform the procedures required by the protocol wherever testing takes place (i.e., indoor laboratory or field station).

9. *Section 792.53 Administrative and personnel facilities.* As in the revised FDA GLP regulations, EPA proposes to delete § 792.53 in its entirety. EPA agrees with FDA that the requirements of this section are not necessary for achieving the goals of the TSCA GLP standards.

10. *Section 792.61 Equipment design.* In § 792.61, EPA proposes to delete the phrase "Automatic, mechanical, or electronic" from the beginning of the first sentence. EPA agrees with FDA that the deletion of these qualifying terms provides for a more general interpretation of the word "equipment."

11. *Section 792.63 Maintenance and calibration of equipment.* a. Consistent with the FDA GLPs, EPA is proposing to amend § 792.63(b) to state that standard

operating procedures (SOPs) for remedial action for equipment, in the event of failure or malfunction of equipment, need only be established when "appropriate." This change acknowledges that laboratories may choose to discard rather than repair equipment, and in such cases SOPs which delineate remedial action are not necessary.

b. EPA is also proposing to conform to the revised FDA GLP regulations by deleting from § 792.63(b) the provision that copies of the SOPs shall be made available to laboratory personnel. EPA still believes that laboratory personnel must have access to laboratory SOPs; however, since this requirement is clearly stated in § 792.81(c), EPA considers the inclusion of this provision in § 792.63(b) to be redundant.

12. *Section 792.81 Standard operating procedures.* a. In § 792.81(b) (1), (2), (6), (7), and (12), EPA is proposing to replace the term "animal" with the term "test system." As discussed previously in this preamble, this modification is consistent with the broad scope of test systems which may be used in environmental testing. Further, the Agency proposes to extend all the SOP requirements outlined by § 792.81 to environmental testing. For instance, the provisions of proposed § 792.81(b)(11), which require SOPs for the maintenance and calibration of equipment, would apply to procedures for preparation and maintenance of incubators, greenhouses, or growth chambers, currently required under § 792.228(d).

b. In § 792.81(b)(5), EPA is proposing to require that SOPs be established for tests wherever the testing is undertaken, including those conducted in the field. Accordingly, it is proposed that § 792.81(b)(5) read "Laboratory or other tests" (see discussion of "field testing" in Unit I.C. of this preamble).

c. In conformance with FDA's revised GLP regulations, EPA is proposing to delete the list of examples for laboratory manuals and SOPs required to be made immediately available under § 792.81(c). EPA still intends that laboratory areas must have immediately available manuals and SOPs for laboratory procedures being performed. This requirement still includes toxicology, histology, clinical chemistry, hematology, teratology, and necropsy, if applicable. However, this list is not all inclusive and is too broad to serve as a useful guide. For example, this requirement also includes SOPs for the maintenance, repair, and calibration of equipment as described in § 792.63(b).

d. EPA is also proposing to amend the language of § 792.81(c) to clarify that the requirement of this section also applies to field testing facilities. Therefore, it is proposed that § 792.81(c) will read, "Each laboratory *or other study* area shall have immediately available manuals and standard operating procedures relative to the laboratory or field procedures being performed."

13. *Section 792.90 Animal and other test system care.* a. EPA is proposing to retitle § 792.90 from "Animal care" to "Animal and other test system care." As previously stated, testing required by EPA may involve plants, soils, microorganisms, and other test systems, in addition to animals. The proposed title to § 792.90 reflects the broader scope of test systems for which the EPA intends this section to apply.

Further, it is proposed that the provisions for test system care for ecological effects testing, found in § 792.228(e), be incorporated into proposed § 792.90. Specifically, the proposed revision incorporates the requirements of: § 792.228(e)(1) into proposed § 792.90(b), § 792.228(e)(2) into proposed § 792.90(d), § 792.228(e)(3) into proposed § 792.90(e)(1), § 792.228(e)(4) into proposed § 792.90(f), § 792.228(e)(5) into proposed § 792.90(g), and § 792.228(e)(6) into proposed § 792.90(j).

b. EPA proposes to modify § 792.90(b) to provide for the evaluation of a test system's health status, or the appropriateness of the test system for the study, according to acceptable "scientific practice." This section, as proposed, will still require that newly received animals must have their health status evaluated according to acceptable veterinary medical practices. However, EPA recognizes that it may not be appropriate to evaluate the health status of certain test systems (e.g., soil or water) or to require that a plant, microorganism, soil, or water be evaluated according to acceptable veterinary medical practice to determine their appropriateness for a study. EPA is only concerned that test systems used in a study are free of any disease or condition which may interfere with the purpose or conduct of the study, and that the proper precautions, as stated in § 792.90(b), are taken to comply with this requirement.

c. Additionally, EPA is proposing to modify § 792.90(b), to require "isolation" rather than "quarantine" of newly received animals. This proposal is consistent with FDA's revision to its GLP regulations.

As previously stated, the intent of § 792.90(b) is to prevent the entry of unhealthy or inappropriate test systems into the study, as required by § 792.90(c). Currently, § 792.90(b) provides that this intent be achieved through "quarantine." However, the term "quarantine" suggests a rigid set of procedures, including a mandatory holding period, a specific list of diagnostic procedures, and the use of specialized facilities and test system care practices, which may be an unnecessary burden to industry.

EPA agrees with FDA's conclusion, discussed in the preamble to its revised GLP regulation (52 FR 33775; September 4, 1987), that isolation and evaluation of health status are sufficient precautions against contamination of test systems and, therefore, fulfill the intent of this section. FDA further states that such a revision would provide laboratories the flexibility to develop isolation and health status evaluation procedures best suited for the age, species, class, and type of the test system, as well as the type of study to be performed.

d. EPA proposes to conform to the FDA GLPs by modifying § 792.90(c) to require isolation of diseased test systems only when necessary.

Currently, § 792.90(c) requires that animals which contract a disease or condition shall be isolated in all cases. This requirement would in turn require that separate facilities be available for the isolation of these animals. However, as discussed in the proposal for § 792.43(c), both EPA and FDA believe that laboratories should be given flexibility in their disposition of diseased test systems. As FDA discussed in the proposed revisions to its GLP regulations (49 FR 43533; October 29, 1984), the proposed modification to § 792.90(c) will allow laboratories the option of: (1) Leaving the diseased test system in the experiment provided that the integrity of the study will not be adversely affected by this action; (2) disposing of the test system; or (3) isolating, treating, and returning the test system to the study.

14. *Section 792.105 Test, control, and reference substance characterization.* a. In revised 21 CFR 58.105(a), FDA has deleted the requirement that test and control substance characteristics shall be determined and documented for each batch "before the initiation of the study." This change has not been incorporated by EPA in its proposed revision to § 792.105(a). However, EPA proposes to modify § 792.105(a) to require that test, control, *or reference* substance characterization be determined and documented for each batch before its use in the experiment. EPA feels that this proposed requirement is necessary because it is essential that characteristics of test, control, and reference substances be known prior to their administration or use in an experiment.

EPA's recent experience with antimony trioxide has shown that extensive analytical work was necessary prior to test initiation. Certain assumptions regarding the product's characteristics were used in the protocols for antimony trioxide testing which proved invalid. These invalid assumptions necessitated modifications to the proposed study, resulting in the delay and rescheduling of other subsequent studies. If the analytical work had preceded the toxicology studies, the studies would not have failed and modifications to the studies would not have been necessary. The Agency's conclusion is that it is better to delay study schedules than to initiate improper experimental procedures which will produce invalid results.

b. FDA has modified 21 CFR 58.105(b) to provide for the determination of the stability of the test or control substance either before the initiation of the study or through periodic analysis of each batch according to written standard operating procedures. EPA has chosen not to adopt this approach in proposed § 792.105(b) because the Agency does not agree that stability can adequately be demonstrated by periodic analysis without initial evaluation.

Further, there are many studies required by EPA where solubility of the test, control, and reference substance is of critical importance, such as aquatic toxicity studies. Therefore, EPA is proposing that solubility of the test, control, and reference substance be determined before the experimental start date if knowledge of the solubility characteristics is relevant for the proper conduct of the experiment.

It is EPA's contention that both stability and solubility of the test, control, and reference substance need to be determined before the experimental start date in order to ensure proper handling and administration of the test substance to the test system. However, since the determination of the solubility of the test, control, and reference substance is not a requirement in FDA's GLP regulations, EPA is interested in receiving public comment on this issue.

15. *Section 792.113 Mixtures of substances with carriers.* a. FDA has modified 21 CFR 58.113(a)(2) to require determination of the stability of the test and control substance in a mixture, as required by the conditions of the study, either before the initiation of the study or through periodic analysis of each batch. While EPA does not propose to modify § 792.113(a)(2) to provide the option of determining the stability of the mixture either before study initiation or through periodic analysis (see discussion for § 792.105(b)), EPA will modify this section to require stability testing only to the extent required by the conditions of the experiment. As proposed for § 792.105(b), EPA is also proposing to require that, when appropriate to the conduct of the experiment, solubility of the test, control, or reference substance in the mixture must be determined in the same manner (see discussion for § 792.105(b)). Additionally, as proposed for § 792.105 (a) and (b), EPA is proposing to replace the phrase "before the initiation of the study" with the phrase "before the experimental start date" (see discussion for § 792.105(a)).

The phrase "as required by the conditions of the experiment" has been added in order to clarify that determination of stability and, if appropriate, solubility of a test, control, or reference substance in a mixture is only necessary to support its actual time of use in the experiment. Therefore, it is not necessary to provide data which illustrate long-term stability of a mixture when the actual time that the mixture is used is short-term. For example, a test, control, or reference substance in a mixture that will be used the same day it is prepared will only require data sufficient to show stability and, if appropriate, solubility for 1 day.

b. Additionally, EPA proposes to incorporate into § 792.113(a)(2), the provision currently found in § 792.228(f)(2), which states that the determination of the stability or solubility of the test, control, or reference substance in the mixture must be done under the environmental conditions specified in the protocol.

c. EPA proposes to add new paragraph (c) to § 792.113 which incorporates the provisions of § 792.228(f)(3).

16. *Section 792.120 Protocol.* a. In 21 CFR 58.120(a), FDA has replaced the qualifying phrase "but shall not necessarily be limited to" with the phrase "as applicable." EPA proposes to adopt FDA's approach with some modifications. It is proposed that the phrase "Where applicable" appear before the information specified in § 792.120(a)(9), and continue to appear before the information required by § 792.120(a)(6). The phrase "but shall not necessarily be limited to" would remain in this section.

In FDA's discussion of this proposal (49 FR 43533; October 29, 1984), concerns were expressed that some of the information required to appear in the protocol is not applicable to all types of testing. Specifically, FDA points to the information required by 21 CFR 58.120(a) (9) and (11). In 21 CFR 58.120, paragraph (a)(9) requires a description of the diet used in a study as well as solvents, emulsifiers, and/or other materials used to solubilize or suspend the test or control substance before mixing with the carrier. FDA points out that this requirement is not applicable to radiation-emitting products. Section 58.120(a)(11) specifies that the protocol shall specify dosage level, and this requirement is not applicable to implantable medical devices.

Clearly, the basis for FDA's change is to accommodate concerns that are specific to the types of testing required by FDA and do not necessarily apply to testing required by EPA. Further, EPA is concerned that placing the phrase "as applicable" in § 792.120(a) suggests that there may be cases where it is not applicable for any of the other information required by § 792.120(a) to appear in the protocol. Therefore, the phrase "as applicable" should only appear before those items which are not necessarily appropriate to appear in the protocol for certain types of testing.

For example, there may be testing required by EPA where it may not be appropriate to require a protocol to contain the information specified in § 792.120(a)(9), such as describing and/or identifying the diet of a human subject involved in exposure testing. Therefore, EPA proposes to add the phrase "Where applicable" before the information specified in proposed § 792.120(a)(9).

b. In 21 CFR 58.120(a)(4), FDA has deleted the requirement that the protocol contain "The proposed starting and completion dates." EPA is proposing to retain this requirement in § 792.120(a)(4), but is proposing to modify this paragraph to require, "The proposed experimental start and termination dates."

EPA believes that this information is necessary for the evaluation of a protocol and the Agency's scheduling of additional related studies and audit reviews. Section 792.120(a)(4) is related to the selected study method, laboratory, and specialist availability, and other Agency and industry priorities. Often a group of experiments are carried out in sequence, so that both start and termination dates affect subsequent study expectations and timetables. Projected experimental start and termination dates identify the normal duration for a given experiment type and reflect any special considerations that may be unique to a laboratory, anticipated analytical or methodology work, and available resources, and it may also affect pending regulatory timetables.

Given that there are hundreds of studies that EPA must track, these estimated schedules, combined with those from other studies, allow the Agency to more efficiently schedule audits and regulatory action. Further considerations are the following: (1) The availability of composite schedules for many studies may be necessary to set realistic regulatory action goals; (2) composite study schedules are evaluated to schedule audits while several studies are ongoing or recently completed, and which may all be at a given laboratory or geographic location. This directly reduces EPA resources necessary for audit and regulatory review functions; and (3) standard business management by objectives requires intermediate calendar goals when scheduling multiple outputs, or a long-term single product. The master on-site laboratory schedule will incorporate these dates to carry out the study.

c. In 21 CFR 58.120(a)(5), FDA has deleted the requirement that the protocol contain a justification for the selection of the test system. EPA has chosen to retain this requirement in proposed § 792.120(a)(5).

Environmental studies, including both ecological effects and chemical fate, are more diverse than health effects testing. Further, details relevant to the test system design are more chemically dependent in the case of environmental effects and chemical fate testing than in the case of health effects testing. Many of the test systems in environmental studies must be modified in accordance with specific chemical characteristics. Therefore, EPA must allow a much broader range of flexibility in the nature of tests and selection of test systems. In order to fully understand the test and its results, EPA needs to have a discussion of the reasons for selection of the test system. In addition, EPA recognizes that industry may be engaged in state-of-the-art environmental testing. Under proposed § 792.120(a)(5), EPA can keep abreast of industry advances in such testing and ensure that their use of test systems is appropriate. EPA is interested in receiving public comment on whether to limit the requirement that the protocol contain a justification of the test system to environmental testing.

d. FDA has deleted from 21 CFR 58.120(a)(10) the requirement that the protocol include the route of administration and the reason for its choice. EPA has chosen to retain this requirement in proposed § 792.120(a)(10).

The chemicals regulated by FDA will usually have a predefined route of exposure. Therefore, it makes sense for FDA to eliminate the requirement to stipulate the route of administration and the reason for its choice within the protocol. Unlike FDA, EPA is concerned with presence in or exposure to various media (i.e., air, water, soil, sediment, chemicals, etc.) and may not know in advance the routes of exposure for the chemicals it regulates. Most chemicals and products regulated by EPA do not have set routes of exposure and may even have multiple routes of exposure. Therefore, EPA must consider a wide range of possible exposure routes in its regulatory decisions. Further, the route of administration is essential to determine the effectiveness of a test system for the purposes of a specific toxicology study. The route of administration affects the real dosage rates, and therefore, affects whether the impact of the exposure of the test substance is acute or chronic.

Therefore, EPA believes that, for its purposes, it is essential that the protocol contain the route of administration and the reason for its choice. This requirement will therefore remain in the EPA's TSCA GLP standards in § 792.120(a)(10).

e. EPA proposes to delete current § 792.120(a)(12) in its entirety. Currently, § 792.120(a)(12) requires that the protocol contain the method by which the degree of absorption of the test and control substance by the test system will be determined. EPA agrees with FDA's conclusion that this requirement is not necessary in the protocol.

f. In proposed § 792.120(a)(14), redesignated from current paragraph (a)(15), EPA proposes to conform with FDA's revised GLP regulations and require that the study director's signature be dated on the protocol.

EPA is proposing in § 792.3 that the study initiation date be defined as the date the protocol is signed by the study director. It is through the proposed requirement of § 792.120(a)(14), that the Agency will be able to identify the official study initiation date.

17. *Section 792.130 Conduct of a study.* a. FDA has modified 21 CFR 58.130(d) to provide that records of gross findings for a specimen from postmortem observations "should" be made available to the pathologist when examining that specimen's histopathology. EPA has chosen to retain the requirement that these records "shall," in all cases, be provided to a pathologist during study of the specimen.

EPA agrees with FDA's conclusion that for most studies it is important for the pathologist to have the records of gross findings available when examining a specimen histopathologically. However, it is FDA's contention that replacing the word "shall" with the word "should" will allow the histopathological evaluation of specimens in a "blind" fashion. EPA also recognizes that it may be appropriate for some studies to provide for "blinding" in histopathological evaluation. However, EPA maintains that, when specified by the protocol, the pathologist can accomplish "blinding," without violating § 792.130 by not looking at the records which have been provided. Therefore, it will remain EPA's requirement that the pathologist must have access to the records of gross findings when examining a specimen histopathologically.

b. In conformance with the revised FDA GLP regulations, in § 792.130(e), EPA proposes to replace the terms "computer" and "computer driven" with the term "automated data collection." EPA agrees with FDA that the terms "computer" or "computer driven" do not adequately reflect the data collection and storage technologies currently used by testing facilities. The Agency believes that the proposed term "automated data collection" provides a more appropriate description of the data collection and storage systems available for industry use.

18. *Section 792.135 Physical and chemical characterization studies.* EPA proposes to add § 792.135 in order to specify the provisions of the proposed TSCA GLP standards which will not apply to studies designed to determine the physical and chemical characteristics of a test, control, or reference substance. Most studies designed to determine the physical or chemical characteristics of a test, control, or reference substance rarely involve any modifications to the protocol or experimental design and are usually conducted in an assembly line fashion. Therefore, proposed § 792.135(a) relaxes the requirements of the GLP standards without compromising the quality or integrity of data generated from these studies.

However, in § 792.135(b), EPA is also proposing that the exemptions listed in proposed § 792.135(a) will not apply to studies designed to determine solubility, octanol water partition coefficient, volatility, and persistence of a test, control, or reference substance. These types of physical and chemical characterization studies are more complex in design, execution, and interpretation, and EPA does not believe that it can be assured of the quality and integrity of data generated from these studies without complete GLP compliance.

19. *Section 792.185 Reporting of study results.* In § 792.185(a)(5), EPA is proposing to require that the final report include information relating to the solubility, in addition to stability, of the test, control, or reference substance, if solubility information was important to the conduct of the experiment. This change is consistent with the proposed modifications to §§ 792.105(b) and 792.113(a)(2) (see the preamble discussion of proposed §§ 792.105(b) and 792.113(a)(2)).

20. *Section 792.190 Storage and retrieval of records and data.* a. In § 792.190(a), EPA proposes to conform to the revised FDA GLP regulations by modifying this section to state that specimens obtained from mutagenicity tests and specimens of blood, urine, feces, and biological fluids generated as a result of a study need *not* be retained. EPA is also proposing that § 792.190(a) state that specimens of soil, water, and plants obtained from environmental testing need *not* be retained. EPA agrees with FDA's conclusion that retention of these specimens beyond initial evaluation is burdensome and does not have a significant impact on the quality of a study.

b. As in the revised FDA GLPs, EPA proposes to revise § 792.190(e) by deleting the requirement that study materials which are retained in archives must be indexed specifically by test substance, date of study, test system, and nature of study. EPA agrees with FDA that the intent of this section is to require indexing of materials in such a way as to permit expedient retrieval from archives. EPA does not believe it is necessary to stipulate the specific indexing terms which must be used.

21. *Section 792.195 Retention of records.* a. EPA proposes to delete paragraphs (b)(2) and (3) of § 792.195, redesignate paragraph (b)(1) as (b), and amend paragraph (b) to require a retention period for documentation records, raw data, and specimens of 5 years from the date the results of any study are submitted to the Agency.

Currently, § 792.195(b) requires a retention period for records, raw data, and specimens under paragraph (b)(1) of 10 years following the effective date of the applicable final test rule and, under paragraph (b)(2) of 10 years following the publication date of the acceptance of a negotiated test agreement. This section also recommends a retention period for such materials of 5 years following the date studies are submitted to the Agency under TSCA section 5.

As stated in the preamble to the 1983 TSCA GLP regulation (48 FR 53935; November 29, 1983), EPA believes that it is essential that study records, raw data, and specimens be maintained to provide the Agency with a sufficient period of time to review the study results and implement any appropriate regulatory actions. Further, it is essential that records, raw data, and specimens be available to suppport Agency decisions in case of court challenges to those decisions. However, the Agency sees no reason to vary record retention requirements and has concluded that a record retention period of 5 years from the date the study is submitted to EPA is a sufficient period of time to meet the Agency concerns and goals. Finally, the record retention period proposed in § 792.195(b) is preferable to the timeframes currently required because it is consistent with the requirements currently set forth in the FIFRA GLPs, in 40 CFR 160.195(b)(2), and the FDA Good Laboratory Practice regulations in 21 CFR 58.195(b).

b. In § 792.195, EPA proposes to delete the examples provided in the first sentence of paragraph (c). EPA has proposed this change in conformity with FDA's recent revision because EPA agrees with FDA that these examples do not clarify which materials must be retained from a study and, therefore, are not necessary in this section.

c. EPA is also proposing to modify § 792.195(c) to state that specimens obtained from mutagenicity tests, specimens of soil, water, and plants, and wet specimens of blood, urine, feces, biological fluids, do not need to be retained beyond quality assurance review. This change has been adopted in order to be consistent with the change discussed in proposed § 792.190(a).

d. In new § 792.195(i), EPA proposes to allow records and other "raw data" required by these regulations to be retained either as original records or as true copies, such as photocopies, microfiche, or other accurate reproductions of the original records. This provision would be incorporated in the TSCA GLPs in § 792.195(i) in order to be consistent with the changes to FDA's Good Laboratory Practice regulations.

II. Economic Analysis

The proposal to expand coverage of the TSCA GLP standards to testing conducted in the field is not expected to increase testing costs significantly. Further, the revisions to the TSCA GLP standards which reflect the FDA GLP revisions primarily provide relief from the original GLP standards (ICF 1987). Therefore, these amendments to the TSCA GLPs are not expected to have a significant economic impact on testing under TSCA.

III. Other Regulatory Requirements

A. Executive Order 12291

Under Executive Order 12291, EPA is required to judge whether a rule is a "major" one and is therefore subject to the requirement of a Regulatory Impact Analysis. The proposed amendments of the TSCA Good Laboratory Practice Standards would not be a major rule because they do not meet any of the criteria set forth and defined in section 1(b) of the Order.

B. Regulatory Flexibility Act

The proposed amendments to the TSCA GLP standards are not expected to have a significant impact on a substantial number of small businesses since little or no economic impact is expected from the revision overall.

C. Paperwork Reduction Act

The Office of Management and Budget (OMB) has approved the information collection requirements contained in this proposed rule under the provisions of the Paperwork Reduction Act of 1980, 44 U.S.C. 3501 *et seq.* and has assigned OMB control number 2070–0033. Comments on these requirements should be submitted to the Office of Information and Regulatory Affairs of OMB, marked "Attention: Desk Officer for EPA." The final rule will respond to any OMB or public comments on the information collection requirements.

List of Subjects in 40 CFR Part 792

Good laboratory practices, Laboratories, Environmental protection, Hazardous materials, Chemicals, Recordkeeping and reporting requirements.

Dated: December 8, 1987.

Lee M. Thomas,

Administrator.

Therefore, it is proposed that 40 CFR Part 792 be amended as follows:

PART 792—[AMENDED]

1. The authority citation for Part 792 is revised to read as follows:

Authority: 15 U.S.C. 2603.

2. In § 792.1, by revising paragraphs (a) and (c) to read as follows:

§ 792.1 Scope.

(a) This part prescribes good laboratory practices for conducting studies relating to health effects, environmental effects, and chemical fate testing. This part is intended to ensure the quality and integrity of data submitted pursuant to testing consent agreements and test rules issued under section 4 of the Toxic Substances

Control Act (TSCA) (Pub. L. 94–469, 90 Stat. 2006, 15 U.S.C. 2603 et seq.).

* * * * *

(c) It is the Agency's policy that all data developed under section 5 of TSCA be in accordance with provisions of this part. If data are not developed in accordance with the provisions of this part, the Agency will consider such data insufficient to evaluate the health and environmental effects of the chemical substances unless the submitter provides additional information demonstrating that the data are reliable and adequate.

3. In § 792.3, by removing the alphabetical paragraph designations in paragraphs (a) through (q); by revising the definitions for "Control substance", "Study," and "Test system"; by replacing the term "Test substance or mixture" with "Test substance"; by amending the definition for "Sponsor" by revising paragraph (2) thereunder; and by adding and alphabetically inserting definitions for "Carrier", "Experimental start date", "Experimental termination date", "Reference substance", "Study completion date", "Study initiation date", and "Vehicle", to read as follows:

§ 792.3 Definitions.

* * * * *

"Carrier" means any material (e.g., feed, water, soil, nutrient media) with which the test substance is combined for administration to test organisms.

"Control substance" means any chemical substance or mixture or any other material other than a test substance, feed, or water that is administered to the test system in the course of study for the purpose of establishing a basis for comparison with the test substance for no effect levels.

* * * * *

"Experimental start date" means the first date the test substance is applied to the test system.

"Experimental termination date" means the last date on which data are collected directly from the study.

* * * * *

"Reference substance" means any chemical substance or mixture or material other than a test substance, feed, or water that is administered to or used in analyzing the test system in the course of a study for purposes of establishing a basis for comparison with the test substance for known effect levels.

* * * * *

"Sponsor" means:

* * * * *

(2) A person who submits a study to the EPA in response to a TSCA section 4(a) test rule and/or a person who submits a study under a TSCA section 4 testing consent agreement or a TSCA section 5 rule or order to the extent the agreement, rule or order references this part; or

* * * * *

"Study" means any experiment in which a test substance is studied in a test system under laboratory conditions or in the environment to determine or help predict its effects, metabolism, environmental and chemical fate, persistence, or other characteristics in humans, other living organisms, or media. The term does not include basic exploratory studies carried out to determine whether a test substance has any potential utility.

"Study completion date" means the date the final report is signed by the study director.

* * * * *

"Study initiation date" means the date the protocol is signed by the study director.

* * * * *

"Test substance" means a substance or mixture administered or added to a test system in a study, which substance or mixture is used to develop data to meet the requirements of a TSCA section 4(a) test rule and/or is developed under a TSCA section 4 testing consent agreement or section 5 rule or order to the extent the agreement, rule or order references this part.

"Test system" means any animal, plant, microorganism, chemical or physical matrix (e.g., soil or water), or subparts thereof, to which the test, control, or reference substance is administered or added for study. "Test system" also includes appropriate groups or components of the system not treated with the test, control, or reference substance.

* * * * *

"Vehicle" means any agent which facilitates the mixture, dispersion, or solubilization of a test substance with a carrier.

4. In § 792.12, by revising the introductory text to read as follows:

§ 792.12 Statement of compliance or non-compliance.

Any person who submits to EPA a test required by a testing consent agreement or a test rule issued under section 4 of TSCA shall include in the submission a true and correct statement, signed by the sponsor and the study director, of one of the following types:

* * * * *

5. In § 792.17, by revising the introductory text of paragraph (a) and paragraph (c) to read as follows:

§ 792.17 Effects of non-compliance.

(a) The sponsor or any other person who is conducting or has conducted a test to fulfill the requirements of a testing consent agreement or a test rule issued under section 4 of TSCA will be in violation of section 15 of TSCA if:

* * * * *

(c) If data submitted to fulfill a requirement of a testing consent agreement or a test rule issued under section 4 of TSCA are not developed in accordance with this part, EPA may determine that the sponsor has not fulfilled its obligations under section 4 of TSCA and may require the sponsor to develop data in accordance with the requirements of this part in order to satisfy such obligations.

6. In § 792.29, by revising paragraphs (d), (e), and (f) to read as follows:

§ 792.29 Personnel.

* * * * *

(d) Personnel shall take necessary personal sanitation and health precautions designed to avoid contamination of test, control, and reference substances and test systems.

(e) Personnel engaged in a study shall wear clothing appropriate for the duties they perform. Such clothing shall be changed as often as necessary to prevent microbiological, radiological, or chemical contamination of test systems and test, control, and reference substances.

(f) Any individual found at any time to have an illness that may adversely affect the quality and integrity of the study shall be excluded from direct contact with test systems, test, control, and reference substances and any other operation or function that may adversely affect the study until the condition is corrected. All personnel shall be instructed to report to their immediate supervisors any health or medical conditions that may reasonably be considered to have an adverse effect on a study.

7. In § 792.31, by revising paragraph (b) to read as follows:

§ 792.31 Testing facility management.

* * * * *

(b) Replace the study director promptly if it becomes necessary to do so during the conduct of a study.

* * * * *

8. In § 792.35, by revising paragraphs (a) and (b) (1) and (3) and removing paragraph (e) to read as follows:

§ 792.35 Quality assurance unit.

(a) A testing facility shall have a quality assurance unit which shall be responsible for monitoring each study to assure management that the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with the regulations in this part. For any given study, the quality assurance unit shall be entirely separate from and independent of the personnel engaged in the direction and conduct of that study.

(b) * * *

(1) Maintain a copy of a master schedule sheet of all studies conducted at the testing facility indexed by test substance and containing the test system, nature of study, date study was initiated, current status of each study, identity of the sponsor, and name of the study director.

* * * * *

(3) Inspect each study at intervals adequate to ensure the integrity of the study and maintain written and properly signed records of each periodic inspection showing the date of the inspection, the study inspected, the phase or segment of the study inspected, the person performing the inspection, findings and problems, action recommended and taken to resolve existing problems, and any scheduled date for re-inspection. Any problems which are likely to affect study integrity found during the course of an inspection shall be brought to the attention of the study director and management immediately.

* * * * *

9. By revising § 792.41 to read as follows:

§ 792.41 General.

Each testing facility shall be of suitable size and construction to facilitate the proper conduct of studies. Testing facilities which are not located within an indoor controlled environment shall be of suitable location to facilitate the proper conduct of studies. Testing facilities shall be designed so that there is a degree of separation that will prevent any function or activity from having an adverse effect on the study.

10. By revising § 792.43 to read as follows:

§ 792.43 Test system care facilities.

(a) A testing facility shall have a sufficient number of animal rooms or other test system areas, as needed, to ensure: proper separation of species or test systems, isolation of individual projects, quarantine or isolation of animals or other test systems, and routine or specialized housing of animals or other test systems.

(1) In tests with plants or aquatic animals, proper separation of species can be accomplished within a room or area by housing them separately in different chambers or aquaria. Separation of species is unnecessary where the protocol specifies the simultaneous exposure of two or more species in the same chamber, aquarium, or housing unit.

(2) Aquatic toxicity tests for individual projects shall be isolated to the extent necessary to prevent cross-contamination of different chemicals used in different tests.

(b) A testing facility shall have a number of animal rooms or other test system areas separate from those described in paragraph (a) of this section to ensure isolation of studies being done with test systems or test, control, and reference substances known to be biohazardous, including volatile substances, aerosols, radioactive materials, and infectious agents.

(c) Separate areas shall be provided, as appropriate, for the diagnosis, treatment, and control of laboratory test system diseases. These areas shall provide effective isolation for the housing of test systems either known or suspected of being diseased, or of being carriers of disease, from other test systems.

(d) Facilities shall have proper provisions for collection and disposal of contaminated water, soil, or other spent materials. When animals are housed, facilities shall exist for the collection and disposal of all animal waste and refuse or for safe sanitary storage of waste before removal from the testing facility. Disposal facilities shall be so provided and operated as to minimize vermin infestation, odors, disease hazards, and environmental contamination.

(e) Facilities shall have provisions to regulate environmental conditions (e.g., temperature, humidity, photoperiod) as specified in the protocol.

(f) For marine test organisms, an adequate supply of clean sea water or artificial sea water (prepared from deionized or distilled water and sea salt mixture) shall be available. The ranges of composition shall be as specified in the protocol.

(g) For freshwater organisms, an adequate supply of clean water of the appropriate hardness, pH, and temperature, and free of contaminants capable of interfering with the study shall be available as specified in the protocol.

(h) For plants, an adequate supply of soil of the appropriate composition, as specified in the protocol, shall be available as needed.

11. By revising § 792.45 to read as follows:

§ 792.45 Test system supply facilities.

(a) There shall be storage areas, as needed, for feed, nutrients, soils, bedding, supplies, and equipment. Storage areas for feed, nutrients, soils, and bedding shall be separated from areas housing the test systems and shall be protected against infestation or contamination. Perishable supplies shall be preserved by appropriate means.

(b) When appropriate, plant supply facilities shall be provided. These include:

(1) Facilities, as specified in the protocol, for holding, culturing, and maintaining algae and aquatic plants.

(2) Facilities, as specified in the protocol, for plant growth (e.g., greenhouses, growth chambers, light banks).

(c) When appropriate, facilities for aquatic animal tests shall be provided. These include aquaria, holding tanks, ponds, and ancillary equipment, as specified in the protocol.

12. By revising § 792.47 to read as follows:

§ 792.47 Facilities for handling test, control, and reference substances.

(a) As necessary to prevent contamination or mixups, there shall be separate areas for:

(1) Receipt and storage of the test, control, and reference substances.

(2) Mixing of the test, control, and reference substances with a carrier, e.g., feed.

(3) Storage of the test, control, and reference substance mixtures.

(b) Storage areas for test, control, and/or reference substance and for test, control, and/or reference mixtures shall be separate from areas housing the test systems and shall be adequate to preserve the identity, strength, purity, and stability of the substances and mixtures.

13. By revising § 792.49 to read as follows:

§ 792.49 Laboratory operation areas.

Separate laboratory space and other space shall be provided, as needed, for the performance of the routine and specialized procedures required by studies.

§ 792.53 [Removed]

14. By removing § 792.53 *Administrative and personnel facilities.*

15. By revising § 792.61 to read as follows:

§ 792.61 Equipment design.

Equipment used in the generation, measurement, or assessment of data and equipment used for facility environmental control shall be of appropriate design and adequate capacity to function according to protocol and shall be suitably located for operation, inspection, cleaning, and maintenance.

16. In § 792.63, by revising paragraph (b) to read as follows:

§ 792.63 Maintenance and calibration of equipment.

* * * * *

(b) The written standard operating procedures required under § 792.81(b)(11) shall set forth in sufficient detail the methods, materials, and schedules to be used in the routine inspection, cleaning, maintenance, testing, calibration, and/or standardization of equipment, and shall specify, when appropriate, remedial action to be taken in the event of failure or malfunction of equipment. The written standard operating procedures shall designate the person responsible for the performance of each operation.

* * * *

17. In § 792.81, by revising paragraphs (b) (1), (2), (3), (5), (6), (7), and (12) and (c) to read as follows:

§ 792.81 Standard operating procedures.

* * * * *

(b) * * *

(1) Test system room preparation.

(2) Test system care.

(3) Receipt, identification, storage, handling, mixing, and method of sampling of the test, control, and reference substances.

* * * * *

(5) Laboratory or other tests.

(6) Handling of test systems found moribund or dead during study.

(7) Necropsy of test systems or postmortem examination of test systems.

* * * * *

(12) Transfer, proper placement, and identification of test systems.

(c) Each laboratory or other study area shall have immediately available manuals and standard operating procedures relative to the laboratory or field procedures being performed. Published literature may be used as a supplement to standard operating procedures.

* * * * *

18. By revising § 792.90 to read as follows:

§ 792.90 Animal and other test system care.

(a) There shall be standard operating procedures for the housing, feeding, handling, and care of animals and other test systems.

(b) All newly received test systems from outside sources shall be isolated and their health status or appropriateness for the study evaluated. This evaluation shall be in accordance with acceptable veterinary medical practice or scientific practice.

(c) At the initiation of a study, test systems shall be free of any disease or condition that might interfere with the purpose or conduct of the study. If during the course of the study, the test systems contract such a disease or condition, the diseased test systems should be isolated, if necessary. These test systems may be treated for disease or signs of disease provided that such treatment does not interfere with the study. The diagnosis, authorization of treatment, description of treatment, and each date of treatment shall be documented and shall be retained.

(d) Warm-blooded animals, adult reptiles, and adult terrestrial amphibians used in laboratory procedures that require manipulations and observations over an extended period of time or in studies that require these test systems to be removed from and returned to their test system-housing units for any reason (e.g., cage cleaning, treatment, etc.), shall receive appropriate identification (e.g., tattoo, toe clip, color code, ear tag, ear punch, etc.). All information needed to specifically identify each test system within the test system-housing unit shall appear on the outside of that unit. Suckling mammals and juvenile birds are excluded from the requirement of individual identification unless otherwise specified in the protocol.

(e) Except as specified in paragraph (e)(1) of this section, test systems of different species shall be housed in separate rooms when necessary. Test systems of the same species, but used in different studies, should not ordinarily be housed in the same room when inadvertent exposure to test, control, or reference substances or test system mixup could affect the outcome of either study. If such mixed housing is necessary, adequate differentiation by space and identification shall be made.

(1) Plants, invertebrate animals, aquatic vertebrate animals, and organisms that may be used in multispecies tests need not be housed in separate rooms, provided that they are adequately segregated to avoid mixup and cross contamination.

(2) [Reserved]

(f) Cages, racks, pens, enclosures, aquaria, holding tanks, ponds, growth chambers, and other holding, rearing, and breeding areas, and accessory equipment, shall be cleaned and sanitized at appropriate intervals.

(g) Feed, soil, and water used for the test systems shall be analyzed periodically to ensure that contaminants known to be capable of interfering with the study and reasonably expected to be present in such feed, soil, or water are not present at levels above those specified in the protocol. Documentation of such analyses shall be maintained as raw data.

(h) Bedding used in animal cages or pens shall not interfere with the purpose or conduct of the study and shall be changed as often as necessary to keep the animals dry and clean.

(i) If any pest control materials are used, the use shall be documented. Cleaning and pest control materials that interfere with the study shall not be used.

(j) All plant and animal test organisms shall be acclimatized, prior to their use in an experiment, to the environmental conditions of the test.

Subpart F—Test, Control, and Reference Substances

19. By revising the heading for Subpart F to read as set forth above.

20. By revising § 792.105 to read as follows:

§ 792.105 Test, control and reference substance characterization.

(a) The identity, strength, purity, and composition, or other characteristics which will appropriately define the test, control, or reference substance shall be determined for each batch and shall be documented before its use in an experiment. Methods of synthesis, fabrication, or derivation of the test, control, or reference substance shall be documented by the sponsor or the testing facility.

(b) The stability and, when relevant to the conduct of the experiment, the solubility of each test, control, or reference substance shall be determined by the testing facility or by the sponsor before the experimental start date. Where periodic analysis of each batch is required by the protocol, there shall be written standard operating procedures that shall be followed.

(c) Each storage container for a test, control, or reference substance shall be labeled by name, chemical abstracts service number (CAS) or code number, batch number, expiration date, if any, and, where appropriate, storage conditions necessary to maintain the

identity, strength, purity, and composition of the test, control, or reference substance. Storage containers shall be assigned to a particular test substance for the duration of the study.

(d) For studies of more than 4 weeks' duration, reserve samples from each batch of test, control, and reference substances shall be retained for the period of time provided by § 792.195.

(e) The stability of test, control, and reference substances under test conditions shall be known for all studies.

21. In § 792.107, by revising the section heading and introductory text to read as follows:

§ 792.107 Test, control, and reference substance handling.

Procedures shall be established for a system for the handling of the test, control, and reference substances to ensure that:

* * * * *

22. By revising § 792.113 to read as follows:

§ 792.113 Mixtures of substances with carriers.

(a) For each test, control, or reference substance that is mixed with a carrier, tests by appropriate analytical methods shall be conducted:

(1) To determine the uniformity of the mixture and to determine, periodically, the concentration of the test, control, or reference substance in the mixture.

(2) To determine the stability and, when relevant to the conduct of the experiment, the solubility of the test, control, or reference substance in the mixture, before the experimental start date. Determination of the stability and solubility of the test, control, or reference substance in the mixture shall be done under the environmental conditions specified in the protocol and as required by the conditions of the experiment. Where periodic analysis of the mixture is required by the protocol, there shall be written standard operating procedures that shall be followed.

(b) Where any of the components of the test, control, or reference substance carrier mixture has an expiration date, that date shall be clearly shown on the container. If more than one component has an expiration date, the earliest date shall be shown.

(c) If a vehicle is used to facilitate the mixing of a test substance with a carrier, assurance shall be provided that the vehicle does not interfere with the integrity of the test.

23. In § 792.120, by revising paragraph (a) to read as follows:

§ 792.120 Protocol.

(a) Each study shall have an approved written protocol that clearly indicates the objectives and all methods for the conduct of the study. The protocol shall contain but shall not necessarily be limited to the following information:

(1) A descriptive title and statement of the purpose of the study.

(2) Identification of the test, control, and reference substance by name, chemical abstracts service (CAS) number or code number.

(3) The name and address of the sponsor and the name and address of the testing facility at which the study is being conducted.

(4) The proposed experimental start and termination dates.

(5) Justification for selection of the test system.

(6) Where applicable, the number, body weight, sex, source of supply, species, strain, substrain, and age of the test system.

(7) The procedure for identification of the test system.

(8) A description of the experimental design, including methods for the control of bias.

(9) Where applicable, a description and/or identification of the diet used in the study as well as solvents, emulsifiers and/or other materials used to solubilize or suspend the test, control, or reference substances before mixing with the carrier. The description shall include specifications for acceptable levels of contaminants that are reasonably expected to be present in the dietary materials and are known to be capable of interfering with the purpose or conduct of the study if present at levels greater than established by the specifications.

(10) The route of administration and the reason for its choice.

(11) Each dosage level, expressed in milligrams per kilogram of body or test system weight or other appropriate units, of the test, control, or reference substance to be administered and the method of frequency of administration.

(12) The type and frequency of test analyses, and measurements to be made.

(13) The records to be maintained.

(14) The date of approval of the protocol by the sponsor and the dated signature of the study director.

(15) A statement of the proposed statistical method.

* * * * *

24. In § 792.130, by revising paragraphs (d) and (e) to read as follows:

§ 792.130 Conduct of a study.

* * * * *

(d) In animal studies where histopathology is required, records of gross findings for a specimen from postmortem observations shall be available to a pathologist when examining that specimen histopathologically.

(e) All data generated during the conduct of a study, except those that are generated by automated data collection systems, shall be recorded directly, promptly, and legibly in ink. All data entries shall be dated on the day of entry and signed or initialed by the person entering the data. Any change in entries shall be made so as not to obscure the original entry, shall indicate the reason for such change, and shall be dated and signed or identified at the time of the change. In automated data collection systems, the individual responsible for direct data input shall be identified at the time of data input. Any change in automated data entries shall be made so as not to obscure the original entry, shall indicate the reason for change, shall be dated, and the responsible individual shall be identified.

25. By adding § 792.135 to read as follows:

§ 792.135 Physical and chemical characterization studies.

(a) Except as provided in paragraph (b) of this section, the following provisions shall not apply to studies designed to determine physical and chemical characteristics of a test, control, or reference substance:

§ 792.31 (c), (d), and (g)
§ 792.35 (b) and (c)
§ 792.43
§ 792.45
§ 792.47
§ 792.49
§ 792.81(b) (1), (2), (6) through (9), and (12)
§ 792.90
§ 792.105 (a) through (d)
§ 792.113
§ 792.120(a) (5) through (12), and (15)
§ 792.165(a) (5) through (8), (10), (12), and (14)
§ 792.195 (c) and (d).

(b) The exemptions provided in paragraph (a) of this section shall not apply to physical/chemical characterization studies designed to determine solubility, octanol water partition coefficient, volatility, and persistence (such as biodegradation, photodegradation, and chemical degradation studies), and such studies shall be conducted in accordance with this part.

26. In § 792.185, by revising paragraphs (a) (4) and (5) to read as follows:

§ 792.185 **Reporting of study results.**

(a) * * *

(4) The test, control, and reference substances identified by name, chemical abstracts service (CAS) number or code number, strength, purity, and composition, or other appropriate characteristics.

(5) Stability and, when relevant to the conduct of the experiment, the solubility of the test, control, and reference substances under the conditions of administration.

* * * * *

27. In § 792.190, by revising paragraphs (a) and (e) to read as follows:

§ 792.190 Storage and retrieval of records and data.

(a) All raw data, documentation, records, protocols, specimens, and final reports generated as a result of a study shall be retained. Specimens obtained from mutagenicity tests, specimens of soil, water, and plants, and wet specimens of blood, urine, feces, and biological fluids, do not need to be retained beyond quality assurance review. Correspondence and other documents relating to interpretation and evaluation of data, other than those documents contained in the final report, also shall be retained.

* * * * *

(e) Material retained or referred to in the archives shall be indexed to permit expedient retrieval.

28. In § 792.195, by revising paragraphs (b) and (c), and adding paragraph (i), to read as follows:

§ 792.195 Retention of records.

* * * * *

(b) Except as provided in paragraph (c) of this section, documentation records, raw data, and specimens pertaining to a study and required to be retained by this part shall be retained in the archive(s) for a period of at least 5 years following the date on which the results of the study are submitted to EPA.

(c) Wet specimens, samples of test, control, or reference substances, and specially prepared material, which are relatively fragile and differ markedly in stability and quality during storage, shall be retained only as long the quality of the preparation affords evaluation. Specimens obtained from mutagenicity tests, specimens of soil, water, and plants, and wet specimens of blood, urine, feces, biological fluids, do not need to be retained beyond quality assurance review. In no case shall retention be required for longer periods than those set forth in paragraph (b) of this section.

* * * * *

(i) Records required by this part may be retained either as original records or as true copies such as photocopies, microfilm, microfiche, or other accurate reproductions of the original records.

Subpart L—[Removed]

29. By removing Subpart L—*Environmental Testing Provisions*, consisting of §§ 792.225, 792.226, 792.228, and 792.232.

[FR Doc. 87-29512 Filed 12-24-87; 8:45 am]
BILLING CODE 6560-50-M

8.2.2 SUMMARY TOXICITY DATA ON DECONTAMINATED CHEMICAL AGENTS

Table 1. Summary Toxicity Data on Decontaminated Chemical Agents.

Test Samples	Toxicity Data[a]				
	Oral LD50 (Rat)	Reference	Dermal LD50 (Rabbit)	Reference	Inhalation LC50[c,d]
HD (5, 10, or 20% NaOH, pH 7)	> 50 mg/kg	Lab Notebook MN 2646 (103-104)	> 200 mg/kg	Lab Notebook MN 2646 (107)	> 2 mg/liter
HD #1 (CH_3OH/40% NaOH, pH 7)	> 50 mg/kg	Lab Notebook MN 2646 (74-75)	> 200 mg/kg	Lab Notebook MN 2646 (82)	> 2 mg/liter
HD #2 (triton X100, 20% NaOH, pH 7.0)	> 50 mg/kg	Lab Notebook MN 2646 (74-75)	> 200 mg/kg	Lab Notebook MN 2646 (83)	
HD #4 (methyl cellusolve, 20% NaOH)	>50 mg/kg	Lab Notebook MN 2646 (74-75)	> 200 mg/kg	Lab Notebook MN 2646 (83)	
HD ($CHCl_3$/5-20% NaOH, pH 7)	> 50 mg/kg	Lab Notebook MN 2647 (24)	> 200 mg/kg	Lab Notebook MN 2647 (27-28)	
HD (5, 10, or 20% NaOH)	> 50 mg/kg	Lab Notebook MN 2646 (103-104)	> 200 mg/kg	Lab Notebook MN 2646 (107)	
Lewisite (5, 10, or 20% NaOH, pH 7)	> 50 mg/kg	Lab Notebook MN 2646 (103)	> 200 mg/kg	Lab Notebook MN 2646 (108)	> 2 mg/liter
Lewisite ($CHCl_3$/NaOH)	> 50 mg/kg	Lab Notebook MN 2647 (32)	> 200 mg/kg	Lab Notebook MN 2647 (33)	> 2 mg/liter
5% HD/5% Lewisite ($CHCl_3$/NaOH (5, 10, 20%), pH 7)	> 50 mg/kg	Lab Notebook MN 2647 (22)	> 200 mg/kg	Lab Notebook MN 2647 (26-27)	

Table 1. Summary Toxicity Data on Decontaminated Chemical Agents (Continued).

Test Samples	Toxicity Data[a] Oral LD50 (Rat)	Reference	Dermal LD50 (Rabbit)	Reference	Inhalation LC50[c,d]
GB (H_2SO_4, pH 4)	> 50 mg/kg	Lab Notebook MN 2702 (78)	> 200 mg/kg	Lab Notebook MN 2702 (78)	
GB (detoxed, dried salts)	> 50 mg/kg	Lab Notebook MN 2702 (56)	> 200 mg/kg	Lab Notebook MN 2702 (57)	
GB (neutralized - Brine - dried salts)	889 mg/kg (24 hr)[b]	Lab Notebook MN 2646 (14-15)			
	568 mg/kg (14 day)[b]	Lab Notebook MN 2646 (14-15)			
GB (demil 09539)	566 mg/kg (24 hr)[b]	Lab Notebook MN 2646 (68-69)	> 200 mg/kg	Lab Notebook MN 2646 (82)	
	271 mg/kg (14 day)[b]	Lab Notebook MN 2646 (68-69)	> 2 g/kg[b]	Lab Notebook MN 2646 (91)	
GA (detoxified)	< 50 mg/kg	Lab Notebook MN 2702 (66)	> 200 mg/kg	Lab Notebook MN 2702 (66)	
VX (H_2O, pH 4.8)	> 50 mg/kg	Lab Notebook MN 2702 (79)	> 200 mg/kg	Lab Notebook MN 2702 (79)	
VX (detoxed, neutralized dried salts)	> 50 mg/kg	Lab Notebook MN 2702 (56)	> 200 mg/kg	Lab Notebook MN 2702 (57)	
VX (HTH)	> 50 mg/kg	Lab Notebook MN 2702 (118)	> 200 mg/kg	Lab Notebook MN 2702 (119)	

[a] Toxicity tests (oral, dermal, inhalation) per DOT guidelines unless otherwise stated.

[b] Toxicity tests (oral, dermal) per FDA guidelines.

[c] DOT inhalation tests, exposure time = 1 hour.

[d] Owens Memo (SAREA-BL-TE, 9 Sep 74).

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8.2.3 TYPE PROTOCOL 210880360000

TYPE PROTOCOL 210880360000

TITLE: Hazard Evaluation of Decontaminated Liquid Waste at CRDEC

DIRECTORATE/DIVISION: Chemical Research, Development and Engineering Center, Research Directorate, Toxicology Division, Biosciences Branch, Aberdeen Proving Ground, MD 21010-5423

RESPONSIBLE INVESTIGATOR(S):

Principal Investigator: James H. Manthei (21/12/87)
James H. Manthei Date

Co-Investigators: John T. James (21/12/87)
John T. James, Ph.D. Date

Dale H. Heitkamp (21/12/87)
Dale H. Heitkamp Date

Quality Assurance Dir: Dennis W. Johnson (21/12/87)
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Branch Chief: John T. James (21/12/87)
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Acting Chief, Biosciences Branch

Division Chief: Harry Salem (22/12/87)
Harry Salem, Ph.D. Date
Chief, Toxicology Division

Director, Research F. Prescott Ward (22 DEC 87)
Dr. F. Prescott Ward, D.V.M., Ph.D. Date
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MANAGEMENT DATA:

Sponsor: Janis Chase (24/12/87)
Janis Chase Date
Chief, Environmental Quality Office

Protocol Number: 210880360000
Project Number:
Job Order Number:

1. Background.

In January 1986 the State of Maryland passed a regulation listing residues of certain decontaminated chemical surety material (CSM) as hazardous waste. Chemical Research, Development and Engineering Center (CRDEC) then initiated a delisting request for these residues, in that they do not meet the criteria for hazardous waste. CRDEC has tasked Research Directorate to provide both analytical and toxicological data that will support this delisting process.

Chemical Division will be designated to provide final decontaminated and neutralized products to Toxicology Division for a toxic hazard evaluation.

Since there exists several accepted decon procedures for many of the CSMs in question, it may become necessary to test one or more of these decon procedures with each of the CSMs.

To answer the questions posed to CRDEC by the State of Maryland in their 3 September 1987 letter, the following criteria must be met:

a. CRDEC must provide a detailed description of the actual decontamination procedures used on the laboratory materials. This must include a step-by-step outline of the decontamination process, and must identify the decontaminating agent used on a given CSM, the theoretical chemical reaction, the concentration of the decontaminating agent used, the amount of time the reaction is allowed to proceed, and any parameters that influence the degree to which the reaction goes to completion.

b. CRDEC must describe the procedures used to assure that the solutions on which toxicological tests are performed are equivalent to the solutions resulting from the actual decontamination procedures.

c. Finally, CRDEC must describe the protocol for the toxicological testing so that the State of Maryland can determine whether it follows generally accepted practices.

In line with the above questions, this Type protocol describes in detail the tests used by Toxicology Division to verify the decontamination of the CSM in question (Question c above). Toxicology Division will determine by the oral and inhalation route in rats, and by the dermal route in rabbits, that the CSM have been decontaminated to a level less than a Class "B" poison using currently approved test procedures as spelled out in CFR 49[1] (DOT tests). This protocol will be used as a Type protocol so any additional CSM or decon procedures can be evaluated by the same procedures as herein described.

The albino rat and New Zealand White (NZW) albino rabbit are the species of choice for "DOT" testing.

The rat and rabbit are the species of choice for these tests as specified in CFR 49.[1]

2. Hypothesis.

Chemical decontamination of CSM, followed by neutralization and subsequent oral, dermal and inhalation toxicity tests, will show that the original CSM have been decontaminated/deactivated to a toxicity level less than a class "B" poison and are no longer a hazardous substance and can be delisted from the State of Maryland's list of hazardous wastes..

3. Materials.

Test materials will be the decontaminated solutions of agents following their neutralization to pH of 7.0. The initial agents will be as chemically pure as available and they will then be decontaminated with an appropriate

caustic or acid as required, followed by a neutralization procedure. These procedures will be carried out by the Chemical group at CRDEC, and the finalized test samples will be provided to the Toxicology Division for testing. Details of the decontamination procedure, as well as the initial agent chemical purity and the neutralization procedure, along with the final pH, will become part of the final document.

4. Methods.

4.1 Procedure for Rat Oral Toxicity Screen.

A group of 10 young adult Sprague Dawley rats (5 each sex) weighing 200 to 300 gm will be given a single oral dose (intubation into stomach) of 50 milligrams per kilogram of the neutralized test substance. Those substances that produce death in half or more than half of the test group would be considered class"B" poisons as specified in CFR 49.[1]

Detailed test procedures for this oral test involve the procurement of healthy Albino rats at least 7 days prior to start of the test. Upon arrival, rats will be quarantined and housed in a suitable room in Bldg E3222 that is climatically controlled to 70°F ± 3° and a relative humidity of 30-70%. Rats will be maintained on approved certified rodent chow and have both food and water available ad libitum during the quarantine. They will be housed two rats per cage, separated by sex and have hardwood chip bedding available. Stainless steel, sequentially-numbered, ear tags will be used for positive identification.

The night before oral dosage each rat will be fasted, but allowed to have access to water. Food should be removed between 1530 hr and 1630 hr the day prior to testing. On the next morning just prior to dosing, access to water will also be restricted. At the time of dosing each rat will be weighed to the nearest gm and then intubated with 0.050 ml/kg of the test substance using a bulb-tipped 16 gauge stainless steel feeding needle. The needle is carefully inserted into the esophagus, and the substance is injected directly into the stomach. All food and water is then withheld for 6 hours so as not to interfere with the complete absorption of the test substance. Each rat is observed for onset of toxic signs, and any deaths or toxic signs will be recorded for onset time, severity and duration. Since this is a 48 hour test, death is the primary endpoint. Following dosage, animals will be housed individually to prevent animal-to-animal interaction. After 48 hours, each rat will be weighed; those that die will be weighed post-mortem. Final disposition of all survivors will be euthanization by CO_2 inhalation.

Identification of each animal will be maintained by cage card and numbered S.S. ear tag during the test.

4.2 Procedures for Rabbit Dermal Toxicity Screen.

As specified in CFR 49, class "B" poisons are those substances that produce death in half or more than half of a group of 10 young adult rabbits weighing 2.3 to 3.0 kg following continuous dermal contact with the bare skin for 24 hours or less. Specifically, our test procedures will include the use

of a group of 10 young adult New Zealand White rabbits (5 each sex) following a quarantine of 7 days. Rabbits will be housed in single unit approved stainless steel cages and have approved certified rabbit chow and water available ad libitum. Quarantine and housing will be in room 106, Bldg E3222, prior to testing and in room 107, Bldg E3222, following testing. Both these rooms will be maintained at 70°F ± 3° and a relative humidity of 30-70%.

The day prior to testing (18-24 hr), each rabbit will be clipped free of hair on the dorsum (back) (approximately 150 sq cm area) using two small animal electric clippers. One clipper will be fitted with a number 2 blade (first clipping) and the second clipping will be done with a number 40 blade. Clipped hair will be removed immediately by vacuum. Each rabbit is returned to its home cage following the clipping procedure.

The next morning, each rabbit, in groups of 10 (5 each sex) will be weighed to the nearest one-hundredth of a kilogram, its metal ear tag number recorded, and each animal will be tattooed with a black ink sequential number inside the left ear.

In order to apply the test material to the skin, each rabbit will be manually restrained by two individuals, and a 2" by 2", two-layer thick surgical gauze patch will be taped to the skin with hypoallergenic tape. At this time a dose of test substance is applied to the skin under the gauze at a volume of 0.200 ml/kg. The gauze is then immediately covered with polyethylene film which is, in turn, tape secured to the clipped skin with additional hypoallergenic tape to form a semi-occlusive protective covering. This procedure is followed by fitting an Elizabethan collar around each rabbit's neck to prevent the animal's licking or scratching at the test site. After the collar is secured, the rabbit is returned to its home cage and the patch is left intact for 24 hours. Rabbits will have free access to food and water and observation for toxic signs and death will continue for 48 hrs. The test patch is removed after 24 hrs, the skin is gently rinsed with lukewarm water, blotted dry, and the rabbit again returned to its home cage.

After the 48 hr test is completed, all surviving rabbits will be euthanized by intravenous (ear vein) injection with T-61 (0.20 ml/kg).

Toxic signs will be carefully observed and recorded both for onset time and severity, as well as duration. Since these decontaminated substances were prepared from either nerve agents or irritants/vesicants, any residual, non-decontaminated agent, should produce either visible toxic signs or skin irritation.

4.3 Procedures for Rat Inhalation Toxicity Screens.

CFR 49 states that a class "B" poison is a vapor, mist, or dust that when continuously inhaled at a concentration of 2 milligrams per liter or less for 1 hr produces death in half or more than half of a group of 10 white laboratory rats (200 to 300 gm) within 48 hours.

Of the 8 compounds listed in Table 1, only 5 have sufficient vapor pressure to be of concern as inhalation hazards. They will be tested under conditions designed to demonstrate any inhalation hazard. The inhalation

hazard test reccgnizes that both volatility and toxicity affect the hazard potential in the workplace. To address these two areas in the complex decon solution, the experimental protocol has been specified as outlined below:

a. Place 5 young adult rats of one sex (acclimated in the animal room for at least 5 days) in an inhalation chamber of $\leq$ 20 liters volume. For each test compound 10 rats are exposed in 2 groups of 5 each, with males and females exposed to each test material. The apparatus is diagrammed in Figure 1.

b. Draw exposure atmosphere through a 5 cm column of the test liquid.

c. Exposure time is 1 hour and the test liquid must be replenished 30 min into the exposure.

The goal is to show <5 deaths for 48 hr after the exposure. This would indicate less than a class B poison.

It should be noted that this procedure gives a maximal concentration compared to that expected in the laboratory. Attempting to directly generate 2 mg/l of the decon material would be technically difficult and would be irrelevant to the workplace hazard. In addition, no attempt will be made to quantitate chamber contents as this would be expected to be a highly complex mixture undergoing rapid change as the more volatile components of the test solution are exhausted. During exposure the chamber temperature will be maintained at 23 $\pm$ 2°C, and once during each exposure the O_2 content will be checked.

5. Technical Methods.

5.1 Rats.

Rats used for oral dosing will be housed in Bldg E3222, room to be determined (possibly 108), which is climatically controlled to 70°F $\pm$ 3° and a relative humidity of 30-70%. Daylight/dark hours will be controlled by timer on a 12-hour cycle. Rat cages will be standard size polycarbonate, containing hardwood chip bedding. During quarantine, rats will be housed in groups of two, by sex, and have certified rodent chow and water available ad libitum. Bedding will be changed on Mondays, Wednesdays and Fridays and food and water will be checked daily. After testing, rats will be housed individually to prevent animal-to-animal contact and cannibalism. Observation for toxic signs will be continuous on test day, and at least three times on day two, or more often if toxic signs persist. After 48 hours all surviving rats will be euthanized by CO_2 inhalation.

Rats used in the inhalation phase of this study will be housed in room 4 of building E3226. Environmental parameters are as above. Caging will be individually, however. Euthanization will be by CO_2 inhalation.

5.2 Rabbits.

Rabbits will be housed in room 106, building E3222, during quarantine. Each rabbit will be in a single unit stainless steel cage and have approved rabbit chow and water available ad libitum. Food and water are checked daily and cage pans are sanitized on Mondays, Wednesdays and Fridays. The temperature will be automatically controlled to within 70°F ± 3°, and relative humidity, 30-70%; light cycles will be maintained automatically with a 12 hour daylight/12 hour dark cycle. Body weights will be monitored upon arrival, at test time, and then at termination or death, whichever occurs first. Identification will be by sequentially-numbered metal ear tag, as well as an identical number tattooed in black ink on the inner surface of the left ear.

Test procedures will be done in rm 107, building E3222, and this room will be environmentally maintained the same as in room 106. Rabbits will be prepared for testing by clipping a 150 sq cm area on their dorsal area using both a number 2 and a number 40 blade attached to small animal clippers. Elizabethan collars will be worn by each rabbit for the duration of the 24 hr exposure to prevent licking and disturbing the test site. Following test completion, rabbits will be euthanized by intravenous injection (ear vein) of T-61 (0.20 ml/kg).

6. Data Analysis.

Data analysis will involve monitoring and recording the onset and duration of toxic signs, as well as times to death. Those substances producing death in half, or more than half, of each group of 10 animals will be considered as class "B" poisons. Since several of the test starting agents substances may produce irritation, this toxic effect will also be monitored.

7. Compound Purity.

The initial starting compounds that will be decontaminated/deactivated by combining them with either bases or acids will be as pure as available at CRDEC and the decontamination materials will be documented by the chemists and supplied for inclusion in the final document. Also to be included is the pH of the final neutralized product.

8. Data Storage.

Test data will be recorded in official CRDEC notebooks along with any computerized data developed. Ultimately, these will be reported in a technical report and final disposition of the test data will be in the Toxicology Division Archives.

The type of data to be recorded include:

a. Animal species, sex, weight, ear tag number.

b. Animal arrival date, test date, termination date.

c. Complete record of toxic signs observed, as well as time of deaths if they occur.

d. Record of feed used, lot number, brand, manufacturer.

e. Times for inhalation exposure.

f. Complete record of chemical substances used, to include starting purity of the agents, decontamination procedures, and the final neutralization procedure, as well as the final pH.

g. Names of individuals involved in the study, along with their qualifications.

h. Record of facility climatic conditions.

i. Any problems that arise, such as climatic, animal health status during quarantine will be documented.

j. Any changes necessary to the procedures spelled out in this protocol will be documented.

9. Pain Category.

Although these substances are to be detoxified and neutralized, the oral and dermal test procedures in themselves will produce some stress. The oral and dermal tests will therefore be conducted as pain/stress without anesthetic or analgesics. The inhalation tests will produce no significant pain or stress.

10. Euthanasia.

Following the completion of the 48 hour test procedure, rats will be euthanized by inhalation of CO_2 and rabbits will be terminated by intravenous (ear vein) injection of T-61 (euthanasia solution), at a volume of 0.2 ml/kg.

11. Bibliography.

1. Code of Federal Regulations, CFR 49 (Transportation) parts 100 to 177, Section 173.343 Poison B, page 602, October 1, 1986.

12. Coordination.

a. Clinical Pathology: None required

b. Anatomical Pathology: None required

c. Animal Requirements:

(1) Species: Rabbit

Strain: NZW

Total Number: 10 per test

Age and Weight: Young adult, 2.3 to 3.0 kg

Sex: 5 male and 5 female per test

Starting Date: Open

Completion Date: Open

(2) Species: Rat

Strain: Sprague-Dawley

Total Number: 20 per test

Age and Weight: Young adult, 200 to 300 gm

Sex: 10 male and 10 female per test

Starting Date: Open

Completion Date: Open

d. Cost Accounting:

(1) Protocol Number:

(2) Project Number:

(3) Job Order Number:

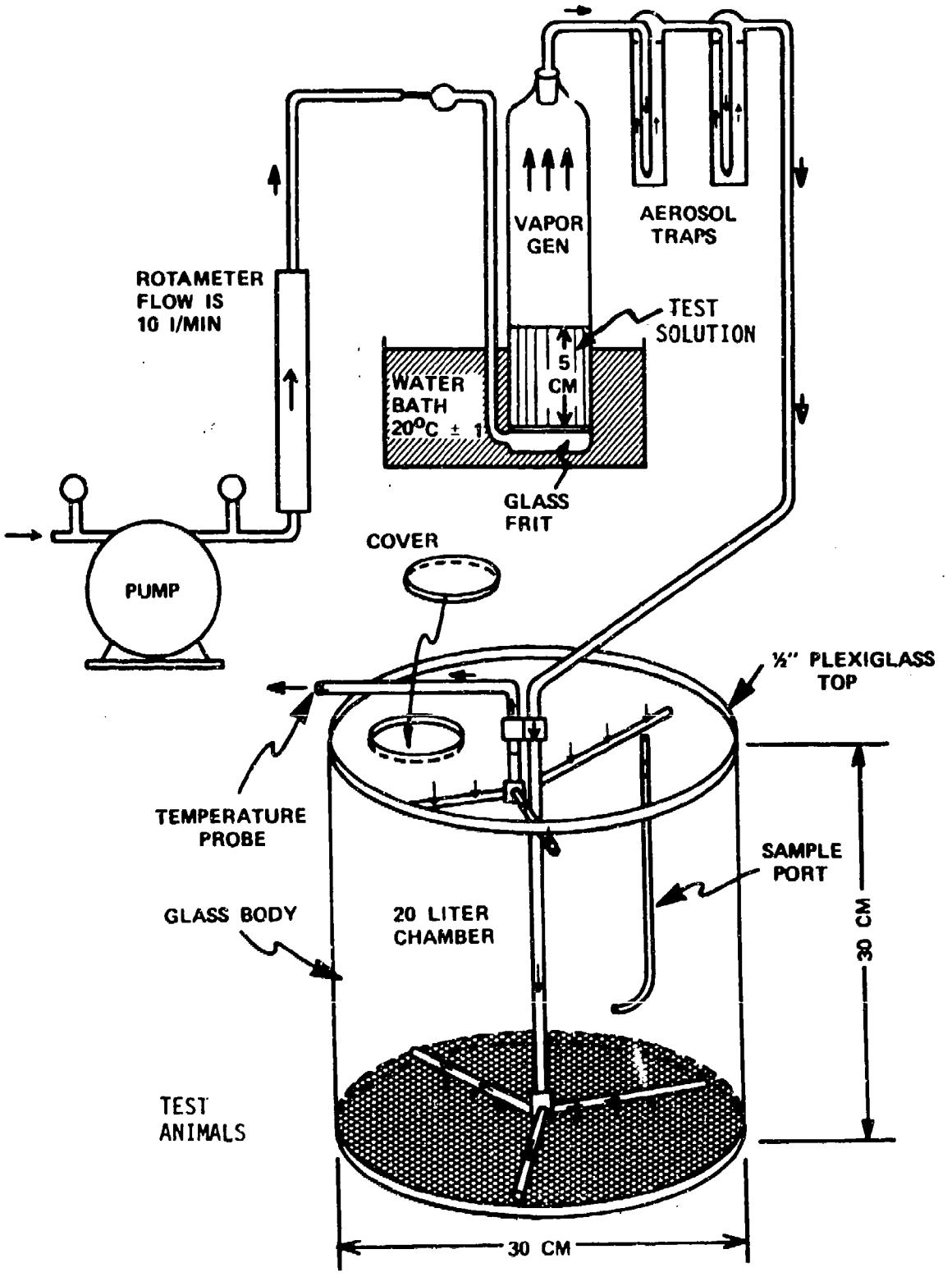

Figure 1. Inhalation Hazard Test of Deconned Agent Solution

8.2.4 CODE OF FEDERAL REGULATIONS DEPARTMENT OF TRANSPORTATION GUIDELINES FOR CLASSES OF POISONOUS MATERIALS

code of federal regulations

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product discharge openings are not permitted.

[Order 60, 30 FR 5745, Apr. 23, 1965, as amended by Order 71, 31 FR 9070, July 1, 1966. Redesignated at 32 FR 5606, Apr. 5, 1967, and amended by Amdt. 173-60, 37 FR 2886, Feb. 9, 1972; 37 FR 3524, Feb. 17, 1972; Amdt. 173-94, 41 FR 16074, Apr. 15, 1976]

Subpart F—Corrosive Materials: Definition and Preparation

SOURCE: 29 FR 18725, Dec. 29, 1964, unless otherwise noted. Redesignated at 32 FR 5606, Apr. 5, 1967.

§ 173.240 Corrosive material; definition.

(a) For the purpose of this subchapter, a corrosive material is a liquid or solid that causes visible destruction or irreversible alterations in human skin tissue at the site of contact, or in the case of leakage from its packaging, a liquid that has a severe corrosion rate on steel.

(1) A material is considered to be destructive or to cause irreversible alteration in human skin tissue if when tested on the intact skin of the albino rabbit by the technique described in Appendix A to this Part, the structure of the tissue at the site of contact is destroyed or changed irreversibly after an exposure period of 4 hours or less.

(2) A liquid is considered to have a severe corrosion rate if its corrosion rate exceeds 0.250 inch per year (IPY) on steel (SAE 1020) at a test temperature of 130° F. An acceptable test is described in NACE Standard TM-01-69.

(b) If human experience or other data indicate that the hazard of a material is greater or less than indicated by the results of the tests specified in paragraph (a) of this section, the Department may revise its classification or make the material subject to the requirements of Parts 170–189 of this subchapter.

[Amdt. 173-61, 37 FR 5947, Mar. 23, 1972; as amended by Amdt. 173-74, 38 FR 20839, Aug. 3, 1973; Amdt. 173-94, 41 FR 16074, Apr. 15, 1976]

§ 173.241 Outage.

(a) The outage (ullage) for packagings containing corrosive liquids, when offered for transportation, must be in accordance with the following requirements:

(1) *General outage requirements.* Packagings must not be completely filled. The proper vacant space (outage) in a tank car or other shipping container depends on the coefficient of expansion of the liquid and the maximum increase of temperature to which it will be subjected in transit. Outage must be calculated to the total capacity of the container.

(2) *Outage requirements for packagings of 110 gallons or less.* Sufficient outage must be provided so that the packaging will not be liquid full at 130° F. (55° C.).

(3) *Outage requirements for tank cars.* In tank cars, outage must be calculated to percentage of the total capacity of the tank, i. e., shell and dome capacity combined. If the dome of the tank car does not provide sufficient outage, then vacant space must be left in the shell to make up the required outage. The outage for tank cars must be not less than 1 percent

(4) *Outage requirements for cargo tanks or portable tanks.* No cargo tank or portable tank, or compartment thereof, used for the transportation of any corrosive liquid shall be completely filled. The outage for cargo tanks and portable tanks must be no less than 2 percent.

[29 FR 18725, Dec. 29, 1964. Redesignated at 32 FR 5606, Apr. 5, 1967, and amended by Amdt. 173-61, 37 FR 5947, Mar. 23, 1972; Amdt. 173-94, 41 FR 16074, Apr. 15, 1976]

§ 173.242 Bottles containing corrosive liquids.

(a) Bottles containing corrosive liquids, as defined by § 173.240, may not be packed in the same outside container with any other article, except as specifically provided in paragraphs (b) and (c) of this section and §§ 173.25, 173.257, 173.258, 173.259, 173.260, 173.261, or 173.286.

(b) Bottles containing corrosive liquids cushioned by incombustible absorbent material and securely packed in tightly closed metal containers, except hydrofluoric acid which must be packed in a container other than a metal container, may be packed with other articles. This exception does not apply to nitric acid exceeding 40 percent concentration, perchloric acid, hydrogen peroxide exceeding 52 percent strength by weight, nitrohydrochloric acid, or nitrohydrochloric acid diluted, which must not be packed in the same outside container with any other article under any circumstances.

[1] Amdt. 173-61, 37 FR 5947, Mar. 23, 1972.

vate and contract motor carriers under conditions specified in § 177.840(a)(1) of this subchapter.

(vii) Pressure in each cylinder must be reduced to 8 psig or lower at least once within 4 hours before the beginning of transportation.

[29 FR 18743, Dec. 29, 1964. Redesignated at 32 FR 5606, and amended by Amdt. 173-6, 34 FR 7161, May 1, 1969; Amdt. 173-94, 41 FR 16081, Apr. 15, 1976]

Subpart H—Poisonous Materials, Etiologic Agents, and Radioactive Materials; Definitions and Preparation

SOURCE: 29 FR 18753, Dec. 29, 1964, unless otherwise noted. Redesignated at 32 FR 5606, Apr. 5, 1967.

§ 173.325 Classes of poisonous materials.

(a) Poisonous materials for the purpose of this subchapter are divided into three groups according to the degree of hazard in transportation.

(1) Poison A.

(2) Poison B.

(3) Irritating material.

[Amdt. 173-94, 41 FR 16081, Apr. 15, 1976]

§ 173.326 Poison A.

(a) For the purpose of Parts 170–189 of this subchapter extremely dangerous poisons, class A, are poisonous gases or liquids of such nature that a very small amount of the gas, or vapor of the liquid, mixed with air is dangerous to life. This class includes the following:

(1) Bromacetone.

(2) Cyanogen.

(3) Cyanogen chloride containing less than 0.9 percent water.

(4) Diphosgene.

(5) Ethyldichlorarsine.

(6) Hydrocyanic acid (see Note 1 of this paragraph).

(7) [Reserved]

(8) Methyldichlorarsine.

(9) [Reserved]

(10) Nitrogen peroxide (tetroxide).

(11) [Reserved]

(12) Phosgene (diphosgene).

(13) Nitrogen tetroxide-nitric oxide mixtures containing up to 33.2 percent weight nitric oxide.

NOTE 1: Diluted solutions of hydrocyanic acid of not exceeding 5 percent strength are classed as poisonous articles, class B (see § 173.343).

(b) Poisonous gases or liquids, class A, as defined in paragraph (a) of this section, except as provided in § 173.331, [illegible] by rail express.

[29 FR 18753, Dec. 29, 1964. Redesignated at 32 FR 5606, Apr. 5, 1967, and amended by Amdt. 173-94, 41 FR 16081, Apr. 15, 1976; Amdt. 173-94A, 41 FR 40683, Sept. [illegible], 1976]

§ 173.327 General packaging requirements for Poison A materials.

(a) Cylinders must be maintained in compliance with the requirements of § 173.34. Valves must be capable of withstanding the test pressure of the cylinders and must have taper-threaded connections directly to the cylinders (no bushings or straight-threaded connections of valves to cylinders permitted). For corrosive commodities, valves may be of the packed type provided the assembly is made gas-tight by means of a seal cap with compatible gasketed joint to the valve body or to the cylinder to prevent loss of commodity through or past the packing; otherwise the valves must be of the packless type with nonperforated diaphragms and handwheels. Each valve outlet must be sealed by a threaded cap or a threaded solid plug. The outlet caps and plugs, luting, and gaskets must be compatible with each other, the valve assembly, and the lading.

(1) The pressure of the poison gas at 130° F. must not exceed the service pressure of the cylinder. Cylinders must not be liquid full at 130° F.

(2) Cylinders packed in boxes must have adequate protection for valves. Box and valve protection must be of strength sufficient to protect all parts of cylinders and valves from deformation or breakage resulting from a drop of at least 6 feet onto a concrete floor, impacting at the weakest point. A cylinder not overpacked in a box must be equipped with a protective cap or other means of valve protection which must be capable of preventing damage to or distortion of the valve if it were subjected to an impact test as follows: The cylinder, prepared as for shipment, is allowed to fall from an upright position with the side of the cap or other valve protection striking a solid steel object projecting not more than 6 inches above the floor level.

(b) Closing and cushioning. All containers must be tightly and securely closed. Inside containers must be cushioned as prescribed, or in any case when necessary to prevent breakage or leakage.

(c) No class A poisons in cargo tanks. No "extremely dangerous poison, class

[29 FR 18753, Dec. 29, 1964. Redesignated at 32 FR 5606, Apr. 5, 1967, and amended by Amdt. 173-73, 38 FR 20085, July 27, 1973; Amdt. 173-94, 41 FR 16082, Apr. 15, 1976]

§ 173.338 [Reserved]

§ 173.343 Poison B.

(a) For the purposes of Parts 170–189 of this subchapter and except as otherwise provided in this Part, class B poisons are those substances, liquid or solid (including pastes and semisolids), other than Class A poisons or Irritating materials, which are known to be so toxic to man as to afford a hazard to health during transportation; or which, in the absence of adequate data on human toxicity, are presumed to be toxic to man because they fall within any one of the following categories when tested on laboratory animals:

(1) *Oral toxicity.* Those which produce death within 48 hours in half or more than half of a group of 10 or more white laboratory rats weighing 300 to 300 grams at a single dose of 50 milligrams or less per kilogram of body weight, when administered orally.

(2) *Toxicity on inhalation.* Those which produce death within 48 hours in half or more than half of a group of 10 or more white laboratory rats weighing 200 to 300 grams, when inhaled continuously for a period of one hour or less at a concentration of 2 milligrams or less per liter of vapor, mist, or dust, provided such concentration is likely to be encountered by man when the chemical product is used in any reasonable foreseeable manner.

(3) *Toxicity by skin absorption.* Those which produce death within 48 hours in half or more than half of a group of 10 or more rabbits tested at a dosage of 200 milligrams or less per kilogram body weight, when administered by continuous contact with the bare skin for 24 hours or less.

(b) The foregoing categories shall not apply if the physical characteristics or the probable hazards to humans as shown by experience indicate that the substances will not cause serious sickness or death. Neither the display of danger or warning labels pertaining to use nor the toxicity tests set forth above shall prejudice or prohibit the exemption of

[29 FR 18753, Dec. 29, 1964. Redesignated at 32 FR 5606, Apr. 5, 1967, and amended by Amdt. 173-94, 41 FR 16083, Apr. 15, 1976, Amdt. 173-94B, 41 FR 57070, Dec. 30, 1976]

§ 173.344 General packaging requirements for Poison B liquids.

(a) Closing and cushioning. All containers must be tightly and securely closed. Inside containers must be cushioned as prescribed, or in any case when necessary to prevent breakage or leakage.

(b) Packagings containing liquid material may not be completely filled. Outage must be as follows:

(1) For packagings of 110 gallons or less, sufficient outage must be provided so that the packaging will not be liquid full at 130° F. (55° C.).

(2) The proper vacant space (outage) in a tank car or other shipping container depends on the coefficient of expansion of the liquid and the maximum increase of temperature to which it will be subjected in transit. Outage must be calculated to the total capacity of the container.

(3) Liquid poison must not be loaded into domes of tank cars.

(4) In tank cars, outage must be calculated to percentage of the total capacity of the tank, i. e., shell and dome capacity combined. If the dome of the tank car does not provide sufficient outage, then vacant space must be left in the shell to make up the required outage.

(5) The outage for tank cars must not be less than 1 percent.

(6) No cargo tank or compartment thereof used for the transportation of any liquid poison shall be completely filled; sufficient space shall be left vacant in every case to prevent leakage from or distortion of any such cargo tank by expansion of the contents due to rise in temperature in transit, and such free space (outage) shall be sufficient in every case so that such cargo tank shall not become entirely filled with the liquid at 130° F.

[29 FR 18753, Dec. 29, 1964. Redesignated at 32 FR 5606, Apr. 5, 1967, and amended by Amdt. 173-94, 41 FR 16083, Apr. 15, 1976; Amdt. 173-94A, 41 FR 40683, Sept. 20, 1976]

§ 173.345 Limited quantities of Poison B liquids.

(a) Limited quantities of Poison B liquids for which exceptions are per-

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CONTAINING
A CODIFICATION OF DOCUMENTS
OF GENERAL APPLICABILITY
AND FUTURE EFFECT

AS OF October 1, 1986

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the Federal Register

with inert luting or gasket material. Valves must be of stainless steel and the caps, plugs, and valve seats must be of material that will not be deteriorated by contact with nitric oxide or nitrogen dioxide. The tank may not be equipped with any safety relief device.

[29 FR 18753, Dec. 29, 1964. Redesignated at 32 FR 5606, Apr. 5, 1967, and amended by Amdt. 173-73, 38 FR 20085, July 27, 1973; Amdt. 173-94, 41 FR 16082, Apr. 15, 1976; Amdt. 173-52, 46 FR 62458, Dec. 24, 1981; 47 FR 13818, Apr. 1, 1982]

§ 173.343 Poison B.

(a) For the purposes of Parts 170-189 of this subchapter and except as otherwise provided in this part, Class B poisons are those substances, liquid or solid (including pastes and semisolids), other than Class A poisons or Irritating materials, which are known to be so toxic to man as to afford a hazard to health during transportation; or which, in the absence of adequate data on human toxicity, are presumed to be toxic to man because they fall within any one of the following categories when tested on laboratory animals:

(1) *Oral toxicity.* Those which produce death within 48 hours in half or more than half of a group of 10 or more white laboratory rats weighing 200 to 300 grams at a single dose of 50 milligrams or less per kilogram of body weight, when administered orally.

(2) *Toxicity on inhalation.* Those which produce death within 48 hours in half or more than half of a group of 10 or more white laboratory rats weighing 200 to 300 grams, when inhaled continuously for a period of one hour or less at a concentration of 2 milligrams or less per liter of vapor, mist, or dust, provided such concentration is likely to be encountered by man when the chemical product is used in any reasonable foreseeable manner.

(3) *Toxicity by skin absorption.* Those which produce death within 48 hours in half or more than half of a group of 10 or more rabbits tested at a dosage of 200 milligrams or less per kilogram body weight, when administered by continuous contact with the bare skin for 24 hours or less.

(b) The foregoing categories shall not apply if the physical characteristics or the probable hazards to humans as shown by experience indicate that the substances will not cause serious sickness or death. Neither the display of danger or warning labels pertaining to use nor the toxicity tests set forth above shall prejudice or prohibit the exemption of any substances from the provisions of Parts 170-189 of this chapter.

[29 FR 18753, Dec. 29, 1964. Redesignated at 32 FR 5606, Apr. 5, 1967, and amended by Amdt. 173-94, 41 FR 16083, Apr. 15, 1976; Amdt. 173-94B, 41 FR 57070, Dec. 30, 1976]

§ 173.344 General packaging requirements for Poison B liquids.

(a) Closing and cushioning. All containers must be tightly and securely closed. Inside containers must be cushioned as prescribed, or in any case when necessary to prevent breakage or leakage.

(b) Packagings containing liquid material may not be completely filled. Outage must be as follows:

(1) For packagings of 110 gallons or less, sufficient outage must be provided so that the packaging will not be liquid full at 130° F. (55° C.).

(2) The proper vacant space (outage) in a tank car or other shipping container depends on the coefficient of expansion of the liquid and the maximum increase of temperature to which it will be subjected in transit. Outage must be calculated to the total capacity of the container.

(3) Liquid poison must not be loaded into domes of tank cars.

(4) In tank cars, outage must be calculated to percentage of the total capacity of the tank, i. e., shell and dome capacity combined. If the dome of the tank car does not provide sufficient outage, then vacant space must be left in the shell to make up the required outage.

(5) The outage for tank cars must not be less than 1 percent.

(6) No cargo tank or compartment thereof used for the transportation of any liquid poison shall be completely filled; sufficient space shall be left vacant in every case to prevent leakage from or distortion of any such

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AND FUTURE EFFECT

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with inert luting or gasket material. Valves must be of stainless steel and the caps, plugs, and valve seats must be of material that will not be deteriorated by contact with nitric oxide or nitrogen dioxide. The tank may not be equipped with any safety relief device.

[29 FR 18753, Dec. 29, 1964. Redesignated at 32 FR 5606, Apr. 5, 1967, and amended by Amdt. 173-73, 38 FR 20085, July 27, 1973; Amdt. 173-94, 41 FR 16082, Apr. 15, 1976; Amdt. 173-52, 46 FR 62458, Dec. 24, 1981; 47 FR 13818, Apr. 1, 1982]

§ 173.343 Poison B.

(a) For the purposes of Parts 170-189 of this subchapter and except as otherwise provided in this part, Class B poisons are those substances, liquid or solid (including pastes and semisolids), other than Class A poisons or Irritating materials, which are known to be so toxic to man as to afford a hazard to health during transportation; or which, in the absence of adequate data on human toxicity, are presumed to be toxic to man because they fall within any one of the following categories when tested on laboratory animals:

(1) *Oral toxicity.* Those which produce death within 48 hours in half or more than half of a group of 10 or more white laboratory rats weighing 200 to 300 grams at a single dose of 50 milligrams or less per kilogram of body weight, when administered orally.

(2) *Toxicity on inhalation.* Those which produce death within 48 hours in half or more than half of a group of 10 or more white laboratory rats weighing 200 to 300 grams, when inhaled continuously for a period of one hour or less at a concentration of 2 milligrams or less per liter of vapor, mist, or dust, provided such concentration is likely to be encountered by man when the chemical product is used in any reasonable foreseeable manner.

(3) *Toxicity by skin absorption.* Those which produce death within 48 hours in half or more than half of a group of 10 or more rabbits tested at a dosage of 200 milligrams or less per kilogram body weight, when administered by continuous contact with the bare skin for 24 hours or less.

(b) The foregoing categories shall not apply if the physical characteristics or the probable hazards to humans as shown by experience indicate that the substances will not cause serious sickness or death. Neither the display of danger or warning labels pertaining to use nor the toxicity tests set forth above shall prejudice or prohibit the exemption of any substances from the provisions of Parts 170-189 of this chapter.

[29 FR 18753, Dec. 29, 1964. Redesignated at 32 FR 5606, Apr. 5, 1967, and amended by Amdt. 173-94, 41 FR 16083, Apr. 15, 1976; Amdt. 173-94B, 41 FR 57070, Dec. 30, 1976]

§ 173.344 General packaging requirements for Poison B liquids.

(a) Closing and cushioning. All containers must be tightly and securely closed. Inside containers must be cushioned as prescribed, or in any case when necessary to prevent breakage or leakage.

(b) Packagings containing liquid material may not be completely filled. Outage must be as follows:

(1) For packagings of 110 gallons or less, sufficient outage must be provided so that the packaging will not be liquid full at 130° F. (55° C.).

(2) The proper vacant space (outage) in a tank car or other shipping container depends on the coefficient of expansion of the liquid and the maximum increase of temperature to which it will be subjected in transit. Outage must be calculated to the total capacity of the container.

(3) Liquid poison must not be loaded into domes of tank cars.

(4) In tank cars, outage must be calculated to percentage of the total capacity of the tank, i. e., shell and dome capacity combined. If the dome of the tank car does not provide sufficient outage, then vacant space must be left in the shell to make up the required outage.

(5) The outage for tank cars must not be less than 1 percent.

(6) No cargo tank or compartment thereof used for the transportation of any liquid poison shall be completely filled; sufficient space shall be left vacant in every case to prevent leakage from or distortion of any such

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PART II

DEPARTMENT OF TRANSPORTATION

Hazardous Materials Regulations Board

■

CONSOLIDATION OF HAZARDOUS MATERIALS REGULATIONS AND MISCELLANEOUS PROPOSALS

Notice of Proposed Rulemaking

H. Section 173.308 would be added to read as follows:

§ 173.308 Cigarette lighter or other similar device charged with fuel.

(a) In addition to the requirements of § 173.21(d), a cigarette lighter or other similar device charged with butane, a butane mixture, or other gaseous mixture having similar properties must be shipped in accordance with the following:

(1) No more than 2.3 fluid ounces of liquefied gas may be loaded into each device;

(2) The pressure in each device may not exceed 140 p.s.i.g. at 130°F.

(3) The liquid portion of the gas may not exceed 85 percent of the volumetric capacity of each fluid chamber at 60°F.

(4) Each device, including closures, must be capable of withstanding an internal pressure of at least 275 p.s.i.g.

(5) Devices must be overpacked in packaging that is designed or arranged to prevent movement of the device itself.

I. In § 173.314, the word chapter would be amended to read "subchapter" in paragraph (b)(4); paragraphs (b)(5) and (6) would be added to read as follows:

§ 173.314 Requirements for compressed gases in tank cars.

* * * * *

(b) * * *

(5) Each tank car, except series 106A*** or 110A*** containing a flammable compressed gas or flammable compressed gas mixture must be marked with the name of contents (§ 172.101) in accordance with the requirements of § 172.310 of this subchapter or as otherwise approved by the Department.

(6) Each tank car containing anhydrous ammonia or chlorine must be marked "ANHYDROUS AMMONIA" or "CHLORINE," as appropriate, in accordance with the requirements of § 172.310 of this subchapter.

§§ 173.315 and 173.316 [Amended]

J. Sections 173.315 and 173.316 would remain the same as now written except the word chapter would be amended to read "subchapter" each time it appears in the sections.

K. Subpart G would be amended as follows:

Subpart G—Extremely and Highly Toxic Materials, Etiologic Agents, and Radioactive Materials, Definitino ad Preparation

A. Section 173.325 would be amended to read as follows:

§ 173.325 Classes of poisonous materials.

(a) Poisonous materials for the purpose of this subchapter are divided into three groups according to the degree of hazard in transportation.

(1) Extremely toxic materials;

(2) Highly toxic material;

(3) Irritating material.

B. Section 173.326 would be deleted and a new § 173.326 would be added to read as follows:

§ 173.326 Extremely toxic materials; definition.

(a) For the purpose of this subchapter, a substance is considered to be an extremely toxic material if it falls within any one of the following categories when tested on laboratory animals according to the test procedures described in this paragraph:

(1) *Ingestion (oral).* Any material that has a single dose LD_{50}[1] of 5 milligrams or less per kilogram of body weight when administered orally to both male and female white rats (young adults);

(2) *Inhalation.* Any material that has an LC_{50}[1] of 50 parts per million or less by volume of a gas or vapor, or 0.50 milligram or less of mist or dust per liter of air when administered by continuous inhalation for 1 hour to both male and female white rats (young adults). If the material is administered to the animals as a dust or mist, more than 90 percent of the particles available for inhalation in the test must have a diameter of 10 microns or less, provided it is reasonably foreseeable that such concentrations could be encountered by man in transportation;

(3) *Skin absorption.* Any material that has an LD_{50} of 20 milligrams or less per kilogram of body weight when administered by continuous contact for 24 hours with the bare skin of rabbits according to the test procedures described in Appendix I to this part.

(b) If human experience or other data indicate that the hazard of a given material encountered during an accidental exposure in transportation is greater or less than indicated by the data from the specified animal tests, the Board may revise the classification for the specific material.

C. Section 173.326a would be added to read as follows:

§ 173.326a Highly toxic materials; definition.

(a) For the purpose of this subchapter, a substance is considered to be a highly toxic material if it falls within any one of the following categories when tested on laboratory animals according to the test procedures described in this paragraph:

(1) *Ingestion (oral).* Any material that has a single dose LD_{50}[1] of more than 5 milligrams but not more than 50 milligrams per kilogram of body weight when orally administered to both male and female white rats (young adults);

(2) *Inhalation.* Any material that has an LC_{50}[1] of more than 50 parts per million by volume of gas or vapor but not more than 200 parts per million or more than 0.50 milligram, but not more than 2 milligrams of mist or dust per liter of air when administered by continuous inhalation for 1 hour or less to both male and female white rats (young adults). If the product is administered to the animals as a dust or mist, more than 90 percent of the particles available for inhalation in the test must have a diameter of 10 microns or less provided it is reasonably foreseeable that such concentrations could be encountered by man in transportation.

(3) *Skin absorption.* Any material that has an LD_{50} of greater than 20 milligrams but not more than 200 milligrams per kilogram of body weight when administered by continuous contact for 24 hours with the bare skin of rabbits, according to the test procedures described in Appendix I to this Part.

(b) If human experience or other data indicate that the hazard of a given material encountered during an accidental exposure in transportation is greater or less than indicated by the data from the specified animal tests, the Board may revise the classification for the specific material.

D. Section 173.326b would be added to read as follows:

§ 173.326b Irritating materials; definition.

For the purpose of this subchapter, a substance is considered to be an irritating material if it causes reversible local irritant effects on eyes, nose, or throat temporarily impairing a person's ability to function to the degree that he cannot take necessary emergency action in the event of leakage.

E. In § 173.327, the heading and paragraphs (c) and (d) would be amended; paragraph (e) would be added to read as follows:

§ 173.327 General packaging requirements for extremely toxic materials.

* * * * *

(c) The transportation of an extremely toxic material is not permitted if there is any type of interconnection between packagings.

(d) No packaging used for the transportation of any liquid material may be completely filled. Sufficient space must be left empty of liquid to prevent leakage from distortion of the packaging caused by expansion of the contents due to rise in temperature during transportation. This free space must be sufficient in each packaging so that it will not become entirely filled with liquid at 130°F.

(e) Each tank car except series 106A*** and 110A***, must be marked with the name of contents (§ 172.101) in accordance with the requirements of § 172.310 of this subchapter.

F. Section 173.328 would be deleted and a new § 173.328 would be added to read as follows:

§ 173.328 Extremely toxic materials not specifically provided for.

(a) Extremely toxic materials, as defined in § 173.326, other than those for which special packaging requirements are prescribed in this part, must be packaged as follows:

(1) Specification 3[2] or 3D (§ 178.41 of this subchapter). Metal cylinders of not over 125 pounds water capacity (nominal). Gaskets, if used between the pro-

[1] LD_{50}, LC_{50}: That dose (LD) or concentration (LC) which will cause death within 14 days to one half of the test animals.

[2] Use of existing cylinders authorized, but new construction not authorized.